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Vascular Functions: Intervention, Clinical Utility, and Modulation with Free Diving

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Vascular Functions: Intervention, Clinical Utility, and Modulation with Free Diving

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Dedication

To my family, friends, mentors, and all the participants that made this dissertation study possible.

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Vascular Functions: Intervention, Clinical Utility, and Modulation with Free Diving

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Measurements of vascular function can be used to assess the risk of cardiovascular events such as myocardial infarction and stroke due to their informative and noninvasive nature. The overall theme of this dissertation is centered around the measures of vascular function. In the first study, we determined if ambulatory (24-hour) blood pressure measurements could improve endothelial function, a key measure of peripheral vascular function, as acting as ischemic preconditioning. The second study evaluated a new measurement of arterial stiffness, heart-thigh pulse wave velocity (htPWV), that allows noninvasive, minimally intrusive, and user-independent acquisition of pulse wave velocity. In the third study, another key measure of vascular function, carotid arterial compliance, would be augmented during the simulated diving maneuver that is known to produce complex hemodynamic responses. The rationale for this project is that brief periods of ischemic preconditioning improve endothelial function, arterial stiffness is an excellent predictor for morbidity and mortality, and that during the diving maneuver, arterial compliance is likely augmented to buffer increased arterial blood pressure/pulsatile stress and maintain continuous perfusion during bradycardia. Accordingly, the following three specific aims were tested: (1) to determine if 24-hour ambulatory blood pressure monitoring would enhance endothelial function, (2) to evaluate

the agreement of an established measure of arterial stiffness, carotid-femoral pulse wave velocity (cfPWV), with the new measure htPWV (3) and to determine the influence of the simulated free diving maneuver on arterial function.

In the first study, we measured endothelial function using flow-mediated dilation before and after 24-hours ambulatory blood pressure monitoring. We found that ambulatory blood pressure monitoring did not influence endothelium-dependent vasodilation acting via ischemic preconditioning. In the second study, we evaluated the agreement of this new methodology htPWV with the well-established cfPWV. We found that the automatic htPWV method has potential as a screening device for assessing arterial stiffness in a clinical setting due to its agreement and strong correlation with cfPWV. In the third study, we measured the cardiovascular responses that occur during simulated free diving. We found that simulated free diving results in bradycardia, increased mean arterial blood pressure, total peripheral resistance, arterial compliance, carotid blood flow velocity, and decreased cardiac output and atrial stiffness. Taken together, the overall findings from this dissertation study highlights how vascular function can be of use as a biomarker for both researchers and clinicians.

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CHAPTER I. INTRODUCTION

Cardiovascular disease is currently the leading cause of morbidity and mortality in the United States (55). The prognostic utility of arterial blood pressure for cardiovascular disease is well established (129). However, measurements of vascular function can also be used to assess the risk of cardiovascular events (e.g., myocardial infarction and stroke) due to their informative and noninvasive nature. These key measures of vascular function include endothelial function and arterial stiffness/compliance.

Endothelial dysfunction is one of the earliest indicators for the development of atherosclerosis (152). In humans, endothelial function is often measured via flow-mediated dilation (FMD) using ultrasound to access the reactivity of an artery following cuff occlusion. An increase in 1% of FMD results in an 8-13% decreased risk of cardiovascular events (63, 101, 130, 191). In addition to risk stratification, measurements of endothelial function can be used to access the efficacy of interventions (90, 138).

Arterial stiffness is an established measurement of vascular dysfunction (76). Arterial stiffness is often measured via pulse wave velocity (PWV) using arterial tonometers (or pressure transducers) and has been shown to be a strong predictor of cardiovascular disease (76) due to the associated increase in ventricular afterload and end-organ damage (118). Additionally, PWV is increased in the presence of aortic and carotid calcification seen with advanced atherosclerotic plaque development (194). There is substantial evidence that increased arterial stiffness is predictive of cardiovascular disease and mortality (150) although it has yet to become widely used in clinical settings due to its technical nature.

Arterial compliance is important for arterial health as reduced compliance (decreased change in volume of a vessel for a given change of pressure) can increase in the incident pressure waves and reflected pressure waves resulting in elevated systolic blood pressure (114). With decreased buffering capacity of elastic arteries, the microvasculature is exposed to increased pulsatility resulting in injury to the capillary networks and end-organ damage (e.g., brain and kidney) (107). Moreover, systemic arterial compliance increases with exercise (23), as such arterial compliance may be augmented during periods of increased cardiovascular demand to buffer elevated arterial pressure waveforms.

Therefore, a comprehensive investigation of vascular function can be used to evaluate the effectiveness of an intervention on improving cardiovascular health, weigh practicality of clinical usefulness, and study physiology. Three investigations included in this dissertation utilized a variety of vascular function measurements (endothelial function, arterial stiffness, and its inverse arterial compliance) to investigate each of these research areas (an intervention, clinical feasibility, and physiological responses). Overall, this study aimed to demonstrate how vascular function could play a pivotal role in the treatment, clinical prognosis, and understanding of cardiovascular physiology.

CHAPTER II. VASCULAR FUNCTIONS: INTERVENTION, CLINICAL UTILITY, AND MODULATION WITH FREE DIVING: A LITERATURE REVIEW

Blood Pressure Determinants

High blood pressure is a major risk factor for morbidity and mortality worldwide (120). Hypertension is defined as a systolic blood pressure ≥130 mmHg and/or diastolic blood pressure of ≥80 mmHg (25). Hypertension is a result of physiological, lifestyle and genetic factors (112). Unfortunately, more than 90% of hypertension cases are of unknown etiology (considered essential or primary hypertension), while the other 10% (secondary hypertension) are a result of a condition or disease such as renal, cardiovascular, neurological and endocrine diseases (112). Prevention and treatment of blood pressure is of paramount importance. A 10-mmHg reduction in systolic blood pressure reduces the risk of cardiovascular events by 20%, coronary heart disease by 17%, stroke by 27%, heart failure by 28%, and all-cause mortality by 13% (40).

A sedentary lifestyle has become more prevalent in our industrialized society as physical activity has been reduced in many occupations due to automation (19). This increased sedentary behavior increases the risk of hypertension, in general each hour of sedentary time results in a 2% increased risk of hypertension (79). During prolonged sitting, reductions in metabolic demand and blood flow have been shown to increase the accumulation of extravascular fluid in the legs (108) as well as decreased shear stress in the superficial femoral artery (26). This reduction in shear stress leads to downregulation of nitic oxide resulting in greater vasoconstriction and elevated blood pressure (31).

Additionally, epidemiological and family studies have demonstrated a heritability component of blood pressure. For example, genetics play a role in the development of hypertension as a family history including both parents and/or grandparents with a history of high blood pressure increases individuals' risk of hypertension (116). Moreover, twin studies have shown higher agreement of blood pressure in monozygotic than dizygotic twins (42). As much as 30% of blood pressure may be heritable (105), as there are 280 genetic variants that are associated with risk of high blood pressure (123).

Ambulatory Blood Pressure Monitoring

Ambulatory blood pressure monitoring has become the preferred out-of-office measurement as it circumnavigates white coat hypertension and masked hypertension (24). Typically, the ambulatory blood pressure device takes blood pressure readings every 15–30 min during the daytime and 20–60 min at night. This allows for insight into an individual's blood pressure changes throughout a 24-hour period. Typically, individuals who are normotensive have a clear diurnal rhythm of blood pressure, with a substantial drop during the first few hours of sleep and a surge upon waking (126). Ambulatory blood pressure monitoring can pick up abnormalities such as a diminished drop (<10%) or an elevation in blood pressure during sleep. Indeed, individuals with a non-dipping pattern are at increased risk for cardiovascular events (119). Similarly, an excessive morning surge in blood pressure results in increased risk for adverse events (69). Therefore, 24-hour ambulatory blood pressure monitoring has been shown to have better prognostic value than a single office measurement (154).

Endothelial Function

Cardiovascular disease progresses undetected for many years, and the endothelium plays a major role in its progression (170). The endothelium was once considered nothing more than a permeable membrane between blood flow and the outer vascular wall, but it is now considered a critical homeostatic organ for the regulation of vascular tone and structure (179). The endothelium is responsible for detecting changes in hemodynamics (shear stress and wall strain), regulating vascular tone, growth, adhesion, and coagulation (170). Endothelial cells can metabolize L-arginine via endothelial nitric oxide synthase to produce nitric oxide. Nitric oxide exerts a cardioprotective role by relaxing smooth muscle cells, decreasing leukocyte adhesion and migration, muscle cell proliferation, platelet adhesion and aggregation (160). Impairment in endothelial function is evident prior to the development of atherosclerosis and may be a predisposing factor for the development of hypertension and diabetes (136, 137). Additionally, endothelial function measured via flow-mediated dilation has been shown to be inversely correlated to the Framingham risk score for cardiovascular disease (184). Endothelial dysfunction is also linked to inflammation within atherosclerotic plaques increasing the risk for cardiovascular events (4, 52, 135). Thus, endothelial function has been included among markers of organ damage in the European hypertension guidelines (94).

Ischemic Preconditioning

Ischemic preconditioning (IPC) refers to the phenomenon whereby three or four brief periods of ischemia, each followed by tissue reperfusion, confers subsequent protection against the magnitude of tissue injury following ischemia (169). Repeated IPC

can also exert influence on vascular function and health, and both long-term multiple sessions (67, 71) and a single session of IPC enhances endothelial function in humans (110). Indeed, a shear stress intervention, in which 5 seconds of brachial occlusion followed by 10 seconds of rest for 30 minutes 5 times per week for a total of 6 weeks, has been shown to enhance FMD (58). IPC can blunt the myocardial damage following an infarction and can reduce the overall amount of damage (125). Seven days of IPC have been shown to improve both local and systemic endothelial function and microcirculation in healthy young males (67). Furthermore, IPC reduces cardiac cell apoptosis (113), myocardial and vascular damage (70). These improvements are seen in both an early (2 to 3 hours) and late phase (3 to 4 days) (75), with limited evidence showing residual effects up to 8 days post IPC (67). Although IPC typically includes repeated bouts of 5 minutes of ischemia followed by reperfusion, 2 minutes of ischemia and reperfusion is sufficient to induce an immediate protective effect (71). In fact, individuals with suspected myocardial infarctions can benefit from IPC in the ambulance on the way to the hospital by demonstrating better myocardial salvage and decreased adverse cardiac and cerebrovascular events over 4 years of follow-up when compared with normative care controls (16). A protective effect of IPC has been demonstrated via decreased troponin expression (an indicator for heart muscle damage) following coronary artery bypass graft surgery in patients who received IPC for 20 days prior to surgery (81).

IPC has an anti-inflammatory effect by reducing neutrophil adhesion to the endothelium and increasing interleukin-10 (22, 146). Cardioprotective signaling molecules that are released locally during IPC include adenosine, bradykinin, opioids, hydrogen sulfide, and nitric oxide (56, 61, 87, 144, 147). These molecules ultimately

activate protein kinase C and reperfusion injury salvage kinases, which modulates the cardioprotective effects (88). Typically, reperfusion injury limits the production of ATP from the mitochondria leading to cell necrosis. These signaling pathways work on the mitochondria by activating ATP-dependent potassium channels, which provide cardioprotection (56). Moreover, IPC has been shown to increase the expression of endothelial nitric oxide synthase mRNA and signal transducer and activator of transcription 5 (STAT-3) as well as increase the amount of circulating endothelial progenitor cells (81).

Ischemia preconditioning is an effective method to protect against ischemia-induced injury (128). This maneuver of ischemic preconditioning typically involves 3–4 cycles of 5 min of ischemia followed by reperfusion using a blood pressure cuff. In many human cardiovascular research studies, it is common to concurrently measure endothelial function and ambulatory (24-h) blood pressure in the same investigation (47, 89, 138). The ambulatory blood pressure device takes blood pressure readings every 15–30 min during the daytime and 20–60 min at night. Even though the duration of ischemia induced by blood pressure measurements is fairly short, repeated episodes of IPC, that is “increasing in dose”, may provide potent reductions in ischemic myocardial damage (186). Indeed, 2 min of ischemia and reperfusion have been shown to be sufficient to induce an immediate protective effect (172).

Arterial Stiffness

Arterial stiffness refers to the material properties of the arterial wall that have functional influences over blood pressure, blood flow, and the diameter changes with each cardiac cycle. Arterial stiffness can be measured via pulse wave velocity (PWV), which is the speed of the forward pressure wave transmitted from the aorta through the vascular tree (91). PWV provides a segmental value of arterial stiffness and has been validated by the Bramwell Hill equation (18). Arterial stiffness has been shown to provide valuable prognostic information for future cardiovascular diseases and events. Specifically, a 1 m/s increase in carotid-femoral PWV (cfPWV) is associated with a 7% increased risk of cardiovascular events (10). Arterial stiffening increases with advancing age (165) and does not seem to be dependent on atherosclerosis (163). With advancing age, mechanical fraying of elastin structures within the vascular wall from repeated bouts of mechanical stress and crosslinking of collagen fibers from advanced glycation end-products, leads to increased stiffening of the arteries (74, 77, 180, 189). Moreover, with elevated arterial stiffening, pulsatile pressure waves within the blood vessels are generated via cardiac contraction and propagates to the peripheral microvasculature, resulting in damage (175). Additionally, arterial stiffness contributes to the development of hypertension and is an accurate predictor of high blood pressure (174).

Measurements of Arterial Stiffness

There are a number of methods that have been developed to measure arterial stiffness. Most of the measurements are based on one of three models developed to capture arterial stiffness (164). One of the most common is the transmission/propagation model, which utilizes tonometer's or cuffs to measure the time it takes for a pressure wave

to travel from a proximal artery to a distal artery. An example of this measure is cfPWV and is often described as the reference standard measure for arterial stiffness with its plethora of studies demonstrating clinical evidence that link it to mortality (10). Other measurements under this model that are commonly used include brachial-ankle PWV and cardio-ankle vascular index. Brachial-ankle PWV is a simplified method in that it uses air pressure and a volume plethysmograph (cuff based) to measure the PWV between the brachial artery around the upper arm and the ankle. However, brachial-ankle PWV is affected by blood pressure (162). Therefore, cardio-ankle vascular index was developed to encompass arterial stiffness of the thoracic, abdominal, common iliac, femoral, and tibial arteries independent of blood pressure (192), although a recent study has indicated that this measure is not truly independent of blood pressure (84). It is calculated using an electrocardiogram, phonocardiogram, brachial artery, and ankle artery waveforms by measuring PWV via dividing vascular length by the time taken for the pulse wave to travel from the aortic valve to the ankles (148, 192).

The next model of arterial stiffness is the pulsation or distension model, which utilizes ultrasound-derived local artery compliance and distensibility measures. Specifically, this measurement relates pressure changes to diameter changes and provides local assessments of arterial stiffness with the use of a pressure transducer on an artery and ultrasound. Measurements under this model include elastic modulus, Young's modulus, arterial distensibility, and β-stiffness index. Elastic modulus is the pressure change required for theoretical 100% stretch from resting diameter. Young's modulus is elastic modulus per unit area. Arterial distensibility is defined as the relative increase in diameter for a given increase arterial blood pressure, the inverse of elastic

modulus (133). β-stiffness index is the ratio of ln(systolic/diastolic pressures) to relative change in diameter (91).

The last model of arterial stiffness is the Windkessel model (a reservoir that allows arterial wall distension that can maintain diastolic pressure and perfusion via elastic recoil during diastolic periods), which measures systemic arterial compliance via the area method. Systemic arterial compliance is often calculated as the ratio of stroke volume divided by pulse pressure (30, 190).

Clinical Utility and Application of Arterial Stiffness

Arterial stiffening of the central arteries results in hemodynamic consequences, such as increased pulse pressure, decreased shear stress, and elevated pulsatile flow into the microcirculation (176). It has been widely demonstrated that higher cfPWV is associated with increased risk of cardiovascular events and their recurrence (10, 183). In 2007, the European Society of Hypertension and Cardiology proposed a cutoff value of 12 m/s to be used as an indicator of subclinical organ damage (94), eventually this threshold was updated to 10 m/s (178). To measure arterial stiffness in a clinical setting, cfPWV could be recommended since it is recommended in multiple populations with supporting evidence from randomized clinical trials and meta-analyses (10, 183). However, arterial stiffness (cfPWV) has yet to become widely used outside of the research setting due to the technical nature of applying the tonometer and acquiring quality pressure waves at both the carotid and femoral arteries. PWV measurements of other arterial segments such as brachial-ankle PWV or cardio-ankle vascular stiffness index have been utilized in Asian populations, although there is a lack of longitudinal studies in the United States and Europe. These cuff-based measurements are much

easier to use in a clinical setting but currently have less evidence via randomized clinical trials and meta-analyses (10). As such, a new measurement of central arterial stiffness using blood pressure cuffs as opposed to tonometers should be developed to make this measurement more clinically applicable.

Recently, a novel index of arterial stiffness heart-thigh PWV (htPWV) was developed to eliminate the placements of transducers on the carotid and femoral arteries and to make the measurement substantially easier. The new measurement allows noninvasive, minimally intrusive, and user-independent acquisition of central pulse wave velocity. However, there is need to validate the agreement with the reference standard for arterial stiffness (cfPWV) and the new device before it could be recommended for use in a clinical setting, given the potential practicality of implementation for novice users.

The Diving Reflex

The diving reflex is a powerful autonomic response that preserves oxygen and maintains blood flow to vital organs that is triggered by apnea and facial immersion in cold water (122). It is characterized by bradycardia, decreased cardiac output, peripheral vasoconstriction and increased arterial blood pressure (6). The diving reflex was first introduced by Edmund Goodwyn in 1786, but its physiological relevance was recognized following a publication by the French physiologist Paul Bert in 1870 in his work with ducks and other animals during asphyxia (12, 51). The increase in peripheral resistance during the diving reflex redistributes blood flow to the vital organs while limiting oxygen consumption. Furthermore, bradycardia decreases the work of the heart to further limit oxygen consumption. As such, the diving reflex has been described as the “master switch of life” (142). Interestingly, the diving reflex is innate in all vertebrates including diving

mammals, reptiles, birds and has been observed even in fish that demonstrate bradycardia when removed from water (142).

The interacting autonomic control that mediates these cardiovascular responses during the diving reflex are complex and are not fully understood. However, it has been shown that trigeminal nerve activity, peripheral chemoreceptors, and baroreceptors play a major role (54, 85, 124). Additionally, afferent and efferent neuron tracts along with the carotid chemoreceptors are important mediators of the reflex (35). The facial immersion in cold water triggers a neuronal afferent response from the trigeminal nerve (3). The nerve fibers innervating the anterior nasal mucosa and paranasal region are essential for this reflex via chemesthetic trigeminal chemoreceptors (103). These afferent neuronal signals are transmitted to the brainstem, resulting in efferent neuronal signals that activate the sympathetic nervous system (muscarinic M2 receptors) inducing peripheral vasoconstriction and parasympathetic nervous system (vagus nerve) eliciting bradycardia. The carotid chemoreceptors also help to stimulate bradycardia and peripheral vasoconstriction (8). Furthermore, this reflex has been shown to provide diagnostic and clinical applications for individuals with vagal dysfunction, diabetic cardiovascular autonomic neuropathy, baroreflex impairment, and aroxysmal atrial tachycardia (187). Therefore, this section of the literature review discusses the effects of free diving and how the diving reflex can be studied to help further our knowledge of the cardiovascular responses that facilitate this “master switch of life”.

Autonomic Control of Circulation During Exercise

The autonomic nervous system is responsible for mediating cardiovascular adjustments necessary to maintain blood pressure and perfusion to vital organs during exercise and strenuous activities such as free diving (46). These cardiovascular adjustments are accomplished by the parasympathetic and sympathetic nervous system via central command, the exercise pressor reflex, the arterial baroreflex, cardiopulmonary baroreceptors, and arterial chemoreceptors (44).

Central command encompasses descending neural signals from the higher brain center that activates the cardiovascular control center (36, 50), eliciting muscle sympathetic nerve activity (17, 50, 117). At the onset of exercise, a reduction in parasympathetic activity via the vagus nerve contributes to increases in heart rate (134). Additionally, at higher intensity exercise, increases in heart rate and contractility are due to increases in cardiac sympathetic activity as well as epinephrine released via the adrenal medulla (44). Furthermore, there is functional sympatholysis (attenuated vasoconstriction) in the working skeletal muscles and a redistribution of cardiac output to these active tissues to supply oxygen and nutrients and clear metabolites (132). Additionally, during dynamic exercise, the sympathetic nervous system is an important regulatory mechanism for blood pressure responses (29).

The exercise pressor reflex demonstrated by the seminal work of Alam and Smirk (1937) highlights the importance of afferent feedback from skeletal muscle in elevating blood pressure during exercise in humans (1). The exercise pressor reflex is encompassed by group III and IV muscle afferents that provide feedback for mechanical and chemical stimuli within the contracting skeletal muscle (27, 102). The metaboreflex

utilizes acid-sensing ion channel 3 for lactic acid, purinergic 2x receptors for adenosine triphosphate, prostaglandin E2 receptor 4 for prostaglandin, and are redundant as a combined blockade is required to attenuate the exercise pressor reflex (157). These afferents project through the dorsal horn of the spinal cord, then to the ventral lateral medulla and the nucleus solitarii (151). Normally, the group III and IV muscle afferents contribute to the cardiovascular responses (e.g., heart rate and blood pressure) to exercise by increasing their discharge frequency.

The arterial baroreflex provides negative feedback that is necessary for regulating beat-to-beat blood pressure (45, 99). Specifically, the arterial baroreceptor is a neurocardiovascular receptor that is made up of unencapsulated stretch-sensitive nerve endings in the carotid sinus bifurcation and aortic arch that transmit neural impulses to the central nervous system. Subsequently, these afferent impulses to the central nervous system are integrated, and the efferent arm of the reflex projects signals via the sympathetic and parasympathetic branches of the autonomic nervous system. When blood pressure increases, the stretch-sensitive nerve endings increase their firing rate, and efferent sympathetic output is inhibited while parasympathetic output increases (109).

The cardiopulmonary baroreflex is made up of mechanically sensitive receptors in the heart, lungs, and great veins that provide feedback to the medullary vasomotor centers through unmyelinated vagal afferents (96, 182). They respond to changes in central venous pressure and volume (93). Although the role of the cardiopulmonary baroreflex is not completely understood, it does play a functional role during exercise by attenuating sympathetic input. For example, the inhibition of muscle sympathetic nerve

activity during exercise may be a result of increased venous return and elevations in central venous pressure, which stimulates the cardiopulmonary baroreceptors (44).

The arterial chemoreflex is made up of chemically sensitive receptors in the carotid bodies and aortic bodies (98). They sense chemical changes in the bloodstream and send afferent signals to the medullary regions via the carotid sinus and vagus nerve, which help provide respiratory and autonomic control (104). The chemoreflex can restrain exercising blood flow by causing vasoconstrictor sympathetic outflow to skeletal muscle during acute hyperoxia (156).

Free Diving (Diving Mammals and Humans)

In the world's largest animal, the blue whale (Balaenoptera musculus), heart rate decreases from 15 bpm to as low as 2 bpm during diving (49). Moreover, their heart rate is only half the resting value during highly metabolic maneuvers (e.g., lunge feeding) (49). This is possible due to their extremely compliant aortic arch that acts as a Windkessel (the compliant aorta acts a reservoir during systole) and maintains blood flow during extreme bradycardia and reduces pulsatility of blood flow into the distal aorta (82). The aortic arch that acts as a Windkessel allows arterial wall distension that is capable of maintaining diastolic pressure and perfusion to the coronary arteries via elastic recoil during extended diastolic periods (118). Other diving mammals such as the Weddell seal (Leptonychotes weddellii) are capable of diving for longer than an hour. The diving reflex stimulus is strong enough to cause bradycardia for the duration of the dive even during intense exercise (142). In contrast to the effects on heart rate, the effect of submersion on stroke volume in diving mammals have been somewhat inconsistent. For example, some studies demonstrated that stroke volume remains consistent during forced or

trained submersion (15) while others report that stroke volume is decreased by up to 50% (37, 153, 193). In trained harbor seals (Phoca vitulina) during voluntary diving at moderate to high intensity exercise, stroke volume has been shown to decrease when compared with the resting value at the surface (127). Stroke volume likely declines due to decreased venous return and cardiac filling and decreased ejection fraction as a result of reduced inotropy (decreased myocardial contractility) (39, 72).

In humans, free diving activities (apnea-related sports) include spearfishing, underwater photography, underwater hockey and rugby, underwater target shooting and synchronized swimming. Free diving requires the capability to suppress the urge to breath and involves prioritizing blood flow to the brain and heart while using the available oxygen as efficiently and sparingly as possible. Free diving in humans, such as the Japanese female pearl divers called Ama have been diving for seaweeds, shellfish and pearls off the coast of Japan for over 2000 years (60). Ama perform over 100 breath-hold repeated diving maneuvers on a daily basis and continue this tradition into old age (149). Recent studies have aimed to determine whether such substantial stimulus resulted in structural and functional changes in this diving population (159, 168). Indeed, Ama demonstrate reduced systemic arterial stiffness than age-matched sedentary peers (168). Additionally, they also have lower arterial stiffness in more proximal and elastic arterial regions, indicating that Ama have superior reservoir function of the proximal aorta and carotid artery when compared with age-matched sedentary controls from the same region (159). This increased compliance of the aorta is necessary to buffer elevated cardiac pulsations during diving maneuvers.

The cardiovascular responses to diving have previously been studied in laboratory settings. Interestingly, the diving reflex elicits greater bradycardia during dynamic exercise than at rest (11). A study utilizing face immersion, apnea and exercise on a cycle ergometer demonstrated reductions in heart rate and cardiac output and increased systemic vascular resistance in humans, which is a similar response observed in seals (13). Furthermore, these cardiovascular responses during simulated diving have augmented responses in trained divers when compared with non-diver controls (171). Collectively, these studies support the notion that the diving reflex is powerful enough to override sympathetic activity from exercise stimulus. However, a recent study measured cardiovascular responses of free divers in the open sea that went to depths up to 30m and revealed that simultaneous activation of exercise (dynamic phases of diving) and the diving reflex led to an absence of cardiac output reduction to conserve oxygen due to increased stroke volume and only decreased heart rates during resting phases of the dive (97). The authors speculate that the sympathetic discharge during the dynamic phases of the dive overrode the parasympathetic activity.

Individual Components Involved in Free Diving

Apnea. The major physiological response allowing vertebrates to endure a lack of oxygen is the diving reflex (46). Apnea is sufficient stimulus to trigger a decrease in heart rate, although bradycardia is blunted during apnea alone when compared with facial immersion and apnea together (143, 158). During breath holding, there is increased anaerobic metabolism as increases in plasma lactate concentration are observed (6). During apnea, there is also reduced oxygen uptake from the lungs to the blood, thereby conserving lung oxygen reserve (86). Additionally, during breath holding, spleen volume

decreases around 20% resulting in increased hematocrit and hemoglobin in the blood to enhance oxygen transport (9). Interestingly, chronic free divers known as Sea Nomads from South East Asia have larger spleen sizes compared to their closest geographic neighbors, the Saluan (62). Moreover, apneic divers with the highest scores (longer breath hold times) have larger spleen volumes when compared to those with lowest competition scores (140).

Chemoreceptor Stimulation. The carotid body is composed of glomus cells that are chemosensitive and type II cells that play a glial-like role as well as a carotid sinus afferent nerve (48). The carotid body detects and elicits responses to hypoxia to maintain arterial blood pH and partial pressure of carbon dioxide (34). Specifically, these responses include bradycardia, vasoconstriction, and secretion of catecholamines (28, 66). Hypoxia is a necessary stimulus for chemoreceptors to aid in the diving reflex bradycardia, while hypercapnia can attenuate this bradycardia response (85).

Lung Volume. The diving reflex is also modulated by pulmonary stretch receptors by changes in intrapleural pressure and lung volume. For instance, the cardiovascular adjustments that occur during apnea are attenuated by excitation of pulmonary stretch receptors (65). Typically, heart rate increases during inspiration due to parasympathetic withdrawal and decreases during expiration with increased parasympathetic tone (28). During diving, bradycardia is greatest with smaller lung volumes due to a decrease in pulmonary stretch receptor activity (7). Indeed, increased hydrostatic pressure with water immersion has been shown to decrease total lung capacity (20). Moreover, heart rate decreases more when lung volumes are held at 60% when compared with 85% of vital capacity (5). This may be a result of smaller lung volumes having minimal stretch receptor

activity. Alternatively, increased intrathoracic pressure is associated with the greater lung volume (85% compared to 60% of vital capacity), thereby inhibiting venous return that reduces stroke volume and causes elevated heart rate (131).

Respiratory Neuronal Drive. The diving reflex bradycardia is affected by the drive to breathe. Specifically, respiratory drive inhibits carotid body activity from stimulating cardiac vagal activity (38). Breath holding acts to simulate the respiratory neuronal drive possibly by inhibiting feedback from the pulmonary stretch receptors, or when muscle spindle afferents are activated when respiratory muscles fail to shorten (185). Additionally, face immersion in cold water reduces the respiratory neuronal drive (111). However, the drive to breathe eventually overcomes this inhibition and results in involuntary respiratory contractions (185).

Muscle Sympathetic Nerve Activity. During diving, facial cold receptors' input and chemoreceptor stimulation modulate sympathetic nerve activity (155). Facial immersion in cold water triggers muscle sympathetic nerve activity but not with warm water (41). Moreover, hypoxia using breath-holding with 10% oxygen results in greater muscle sympathetic nerve activity than when breath holding with 100% oxygen or room air (80). Additionally, the anticipation to dive increases skin sympathetic nerve activity (41). Chemoreceptor stimulation also helps to cause peripheral vasoconstriction and increases muscle sympathetic nerve activity (155).

Arterial Compliance

Increased arterial compliance is necessary to buffer elevated cardiac pulsations during diving maneuvers (159). It has been demonstrated that blood flow in the carotid

artery is increased during breath-holding and diving (121). This blood flow redistribution via peripheral vasoconstriction during diving maneuvers ensures the availability of oxygen to the brain (121). Moreover, cerebral blood flow is substantially increased during facial immersion in cold water (73). Additionally, apnea augments pial artery pulsations but appears to be independent of cardiac changes indicating a protective mechanism for perfusion to the brain (188). Indeed, free divers coping mechanism against hypoxia is a substantial increase in cerebral blood flow, suggesting cerebral autoregulation is compromised to sustain brain function (181). With increases in arterial pressure and increased perfusion to the brain, it is feasible to speculate that carotid artery compliance is augmented to help buffer these elevated cardiac pulsations during diving maneuvers.

In addition to the diving reflex that is triggered by facial immersion in cold water (122), diving maneuvers are accompanied by apnea and exercise. Each of these stimuli have varying influences on the cardiovascular responses that occur during the diving maneuver. The degrees of diving bradycardia and hypertension induced by the diving reflex are fairly substantial in spite of the vigorous underwater swimming performed by sea divers (97). During free diving, mean arterial pressure has been shown to increase from 96 to 113 mmHg (97). This condition provides a circulatory challenge to properly buffer and cushion cardiac pulsations. Therefore, with increased blood pressure during diving maneuvers, carotid artery compliance may be augmented to help buffer these elevated cardiac pulsations. However, prior to this study, the hemodynamic changes that occur in the vasculature during the diving maneuver have not been investigated.

Conclusion

Each of the three aims discussed above utilize measures of vascular function to evaluate varying research topics. Specifically, this dissertation study investigated an intervention in which ambulatory blood pressure monitoring may act as ischemia preconditioning to enhance endothelial function, evaluated the clinical feasibility of a novel nontechnical measure of central arterial stiffness by comparing its agreement to the reference standard, and studied the cardiovascular responses that occur during the diving reflex to further understand how vital organs maintain perfusion. Taken together, these studies highlight how vascular function is a useful biomarker for both researchers and clinicians.

CHAPTER III. STUDY 1: DOES 24-H AMBULATORY BLOOD PRESSURE MONITORING ACT AS ISCHEMIC PRECONDITONING AND INFLUENCE ENDOTHELIAL FUNCTION[1]

[1] Fico, B. G., Zhu, W., & Tanaka, H. (2019). Does 24-h Ambulatory Blood Pressure Monitoring Act As Ischemic Preconditioning and Influence Endothelial Function? Journal of Human Hypertension, 33(11), 817-820.

Author contributions- Conception and design: Fico B.G., Tanaka H.; Data collection: Fico B.G.; Data analysis and interpretations: Fico B.G., Tanaka H.; Manuscript writing and revisions: Fico B.G., Zhu W., Tanaka H.; Final approval: Fico B.G., Zhu W., Tanaka H.

Abstract

Purpose: Ischemic preconditioning can exert a powerful protection against a subsequent period of ischemia, via repeated inflation and deflation of a blood pressure cuff. Most often, damages of ischemia-reperfusion injury and benefits of preconditioning are evaluated via endothelial function. The ambulatory blood pressure monitoring constitutes repeated bouts of ischemia for 24 h. We examined whether repeated bouts of ischemia accumulated over 24 h influenced endothelial function. Methods: Twenty-two apparently healthy non-medicated middle-aged subjects 41 ± 8 years participated in the study. This study was registered with ClinicalTrials.gov (NCT03303404). Flow-mediated dilation (FMD) was measured as an index of endothelium-dependent vasodilation. Results: The ambulatory blood pressure monitoring device went through an average of 110 ± 13 inflation/deflation cycles, which resulted in 46 ± 6 min of cumulative ischemic stimuli. Following 24-h of ambulatory blood pressure monitoring, FMD did not change significantly 6.6 ± 2.0 vs. 6.8 ± 2.7%. Similarly, shear rate and reactive hyperemia were unchanged. Conclusion: We concluded that ambulatory blood pressure monitoring did not influence endothelium-dependent vasodilation acting via ischemic preconditioning.

INTRODUCTION

Reestablishing blood flow to ischemic organs causes endothelial dysfunction leading to ischemia-reperfusion injury and tissue death. This so-called ischemia-reperfusion injury can be attenuated or prevented by preceding brief periods of ischemia, each followed by tissue reperfusion (169). This maneuver is referred to as ischemic preconditioning (IPC) and typically involves 3-4 cycles of 5 min of ischemia followed by reperfusion using a blood pressure cuff. In many human cardiovascular research studies, it is common to concurrently measure endothelial function and ambulatory (24-h) blood pressure in the same investigation (47). The ambulatory blood pressure device takes blood pressure readings every 15-30 min during the daytime and 20-60 min at night. Even though the duration of ischemia induced by blood pressure measurements is fairly short, repeated episodes of IPC, that is “increasing in dose”, may provide potent reductions in ischemic myocardial damage (186). Indeed, 2 min of ischemia and reperfusion have been shown to be sufficient to induce an immediate protective effect (172). In addition, a shear stress intervention, in which 5 s of brachial occlusion followed by 10 s of rest for 30 min, five times per week, for 6 weeks enhanced flow-mediated dilation (FMD) in healthy males (58).

Prior to this study, there was no information available as to whether ambulatory blood pressure monitoring could induce preconditioning effects and increase endothelial function via repeated bouts of inflation/deflation cycles. As such, we hypothesized blood pressure monitoring would induce preconditioning effects and increase endothelial function.

METHODS

Twenty-two apparently healthy normotensive middle-aged participants came to the laboratory following an overnight fast, participant demographics are presented in Table 1. Prior to testing, participants gave their written informed consent and completed a health history questionnaire. Participants were excluded from the study if they were pregnant, had a recent illness, history of diabetes, heart disease, or other cardiovascular problems, recent surgery, or any other medical intervention. Using the medical history questionnaire our participants reported an average of 2 ± 1 risk factors, including age, family history, smoking, blood pressure, total cholesterol, physical inactivity, psychological stress, high-fat diet, and overweight/obesity. All subjects gave their written informed consent prior to study participation, and the ethics committee of The University of Texas at Austin approved all procedures. This study was approved by the local IRB and registered with ClinicalTrials.gov (NCT03303404).

After 20 min of supine rest, endothelial function was assessed using flow-mediated dilation (FMD; index of endothelium-dependent vasodilation) (32). After another 20 min of rest, a second measure of endothelial function was performed. These two measurements were used to establish repeatability of endothelial function within the laboratory. For the data analyses, only the second measurement of FMD was used as the baseline (pre) measure of endothelial function. Following these procedures, the participants were given an ambulatory blood pressure monitoring device. After 24-h, participants returned to the laboratory, and their endothelial function was reevaluated following 20 min of rest.

Flow-mediated dilatation was measured with a semiautomated diagnostic ultrasound system (UNEX-EF38G, UNEX Corporation, Nagoya, Japan) while participants rested in a supine position (173). A blood pressure cuff was placed on the forearm with the proximal edge of the cuff below the participant's antecubital fossa. The cuff was inflated to 50 mmHg above resting systolic blood pressure for 5 min to occlude blood flow. The position of the probe was marked on each participant to ensure subsequent measurements were taken on the same region of the brachial artery. Shear rate (s^{-1}) was calculated by the following equation: 8 × blood flow velocity (m s^{-1})/internal artery diameter (53).

Ambulatory blood pressure monitoring provides an insight into blood pressure changes in everyday life and an estimate of the overall blood pressure load exerted on the cardiovascular system over 24 h (126). Blood pressure recordings were made using a noninvasive ambulatory monitor (Spacelabs 90227, Spacelabs Healthcare Inc., Redlands, Washington, USA) (90). Participants' arm circumferences were measured to determine appropriate blood pressure cuff sizes; 24–32 cm for adult, 32–42 cm for large adult, and 38–50 cm for extra-large adult cuffs. The cuff was programmed to inflate automatically to 170 mmHg every 15 min from 6 a.m. to 11 p.m. and every 20 min between 11 p.m. and 6 a.m. Physical activity was monitored using a pedometer, which was attached to a belt around the participant's waist. (OMRON HJ-320, OMRON Healthcare Inc., Hoffman Estates, Illinois, USA). The cumulative ischemic stimuli from the ambulatory blood pressure monitoring were calculated by recording the average time the cuff was inflated above the participant's systolic blood pressure and then multiplied by the number of measurements taken.

Statistical analyses were performed with GraphPad Prism (version 7.05, San Diego, California, USA). Data were first tested for the normal distribution. Statistical significance before and after ambulatory blood pressure monitoring for FMD, and blood flow responses were evaluated by the Student's t-tests for paired data. A post hoc power analysis was conducted using the program G*Power (version 3.1.9.2). Considering the difference between the mean and standard deviation (Pre to Post) with an alpha level of 0.05 for FMD in response to ABPM, the overall sample size of 22 participants in this study achieved an adequate power (>80%). A p value < 0.05 was used to determine statistical significance.

Table 1. Participant Characteristics

Variable	***Means ± SD***
Age, years	41 ± 8
Males/Females, n	12 M / 10 F
Height, cm	170 ± 10
Body weight, kg	74 ±17
BMI, kg/m^2	25 ± 4
Heart rate, bpm	71 ± 9
Systolic BP, mmHg	121 ± 24
Diastolic BP, mmHg	74 ± 12
Mean BP, mmHg	89 ± 14
PP, mmHg	47 ± 14
PA, steps	7620 ± 2473
MVPA, bouts/week	4 ± 2

BMI = body mass index; BP = blood pressure; PP= pulse pressure; PA = physical activity; MVPA = moderate to vigorous physical activity.

RESULTS

The ambulatory blood pressure monitoring device went through an average of 110 ± 13 inflation/deflation cycles. Due to erroneous readings, there were an average of 13 ± 6 re-measures. Which resulted in 46 ± 6 min of cumulative ischemic stimuli. Average 24-h systolic and diastolic blood pressure were 121 ± 24 and 74 ± 12 mmHg. The repeated baseline FMD measurements coefficient of variation was 6 ± 9%. We demonstrated FMD 6.6 ± 2.0 vs. 6.8 ± 2.7% was not significantly increased following 24-h of ambulatory blood pressure monitoring ($p = 0.57$) (Figure 1). Similarly, shear rate 88 ± 51 vs. 84 ± 66 s^{-1} and reactive hyperemia 262 ± 216 vs. 320 ± 349 s^{-1} were unchanged ($p = 0.81$, $p = 0.26$).

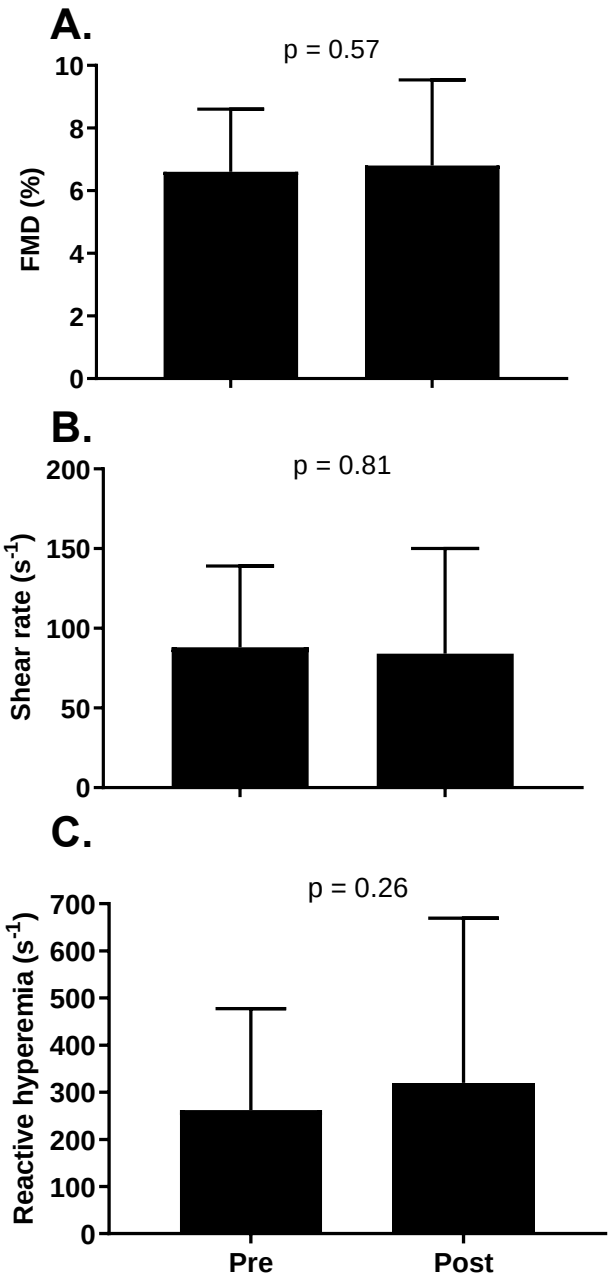

Figure 1. Brachial artery flow-mediated dilation (panel A), shear rate (panel B), and reactive hyperemia (panel C) before (Pre) and after (Post) 24-hour ambulatory blood pressure monitoring stimuli. Data are presented as means ± SD.

DISCUSSION

The purpose of this study was to determine the influence of 24-h ambulatory blood pressure monitoring on endothelial function. We demonstrated that FMD, shear rate, and reactive hyperemia were not influenced by the 24-h ambulatory blood pressure monitoring stimuli.

The present study findings have at least two important implications. First, the 46 ± 6 min of cumulative ischemia via 24-h of blood pressure monitoring in the present study was not sufficient to produce significant changes in endothelium-dependent vasodilation in middle-aged adults. A recent shear stress intervention, involving 5 s brachial occlusion, 10 s rest for 30 min, five times weekly for 6 weeks, enhanced endothelial function (32). In addition, an ischemia preconditioning intervention over two weeks resulted in a robust 2.2% increase in FMD (68). A cuff around the upper arm was inflated to 220 mmHg for 5 min using a rapid inflator to produce an ischemia stimulus, and then deflated to allow reperfusion for 5 min. This cycle was repeated four times per episode (68). The pattern of ischemic stimulus we used might account for the unchanged FMD in the present study. In addition, IPC has been shown to have ‘early’ and ‘late’ phases of protection. Indeed, the previous study observed improvements in FMD during the late phase (68). Our study probed for improvements in endothelial function immediately within the early stage of protection. Thus, future studies are needed to determine if repeated bouts of ambulatory blood pressure monitoring can modulate endothelial function, as clinical benefits of IPC are becoming evident (169). Second, many cardiovascular research laboratories measure 24-h ambulatory blood pressure monitoring and FMD in tandem in the same study (47). Accordingly, it would be of particular interest if 24-h of ambulatory blood

pressure monitoring would influence endothelial function via ischemic preconditioning. In the present study, we did not observe any interference between the measurements. In addition, it should be noted that our findings are limited to apparently healthy adults. As such, future studies are needed to address whether ambulatory blood pressure monitoring stimuli can modify endothelial function in clinical populations.

In conclusion, the present study showed that 24-h of ambulatory blood pressure monitoring did not influence endothelial function acting through ischemic preconditioning. Thus, 24-h ambulatory blood pressure monitoring and FMD would be measured in tandem with no interference.

CHAPTER IV. STUDY 2: HEART-THIGH CUFF PULSE WAVE VELOCITY: A NOVEL NONTECHNICAL MEASURE OF ARTERIAL STIFFNESS[2]

[2] Fico, B. G., Gourley, D. D., Wooten, S. V., & Tanaka, H. (2019). Heart-Thigh Cuff Pulse Wave Velocity: A Novel Nontechnical Measure of Arterial Stiffness. American Journal of Hypertension, 32(11), 1051-1053.

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Abstract

Purpose: Recently, a novel index of arterial stiffness was developed to eliminate the placements of transducers on the carotid and femoral arteries and to make the measurement substantially easier. We evaluated the agreement of this new methodology with the well-established carotid–femoral pulse wave velocity (cfPWV). Methods: A total of 50 adults (28 men and 22 women) varying widely in age and blood pressure were studied. Heart-thigh pulse wave velocity (htPWV) and cfPWV were measured and compared. Results: Mean values of cfPWV and htPWV were 713 ± 145 and 699 ± 150 cm/s and were not significantly different ($P = 0.43$). Both cfPWV and htPWV were significantly associated with age ($r = 0.80$ and 0.58), body mass index ($r = 0.44$ and 0.31), and systolic blood pressure ($r = 0.42$ and 0.41). Conclusion: The two pulse wave velocity measures demonstrated a strong linear association with a Pearson correlation coefficient of 0.64 ($P < 0.001$). This agreement was consistent with the results of the Bland–Altman plot. The automatic htPWV method, which permits the data acquisition with minimum technical skill, time, and intrusion in an operator independent fashion, has potential as a screening device for assessing arterial stiffness in a clinical setting.

INTRODUCTION

Arterial stiffness is a major contributor to cardiovascular disease and is an important element for risk stratification (10). Given the substantial emphasis placed on primary and secondary prevention of cardiovascular disease, a number of noninvasive techniques have been developed to assess vascular stiffening (83, 164). Among them, carotid–femoral pulse wave velocity (cfPWV) has been considered a reference standard measure of arterial stiffness because of the accumulated clinical data and strong associations with a variety of disease states (10). However, this technique requires operator skills to acquire high-fidelity pulse waves on the carotid and femoral arteries as well as the exposure of femoral artery near the groin. Presently, this technique has not been used in routine clinical settings and remains only in the clinical research domain.

Recently, a novel index of arterial stiffness was developed to eliminate the placements of transducers on the carotid and femoral arteries and to make the measurement substantially easier. The new device allows noninvasive, minimally intrusive, and user-independent acquisition of pulse wave velocity. The primary purpose of the present study was to evaluate the agreement of this new methodology with the well-established cfPWV.

METHODS

A total of 50 adults (28 men and 22 women) varying widely in age (18–78 years) and systolic blood pressure (98–166 mm Hg) were studied (Table 2). Participants who had apparent cardiovascular disease were excluded from the study by using a health history questionnaire. All participants gave their written, informed consent to participate

in the study. All procedures were reviewed and approved by The University of Texas at Austin's Institutional Review Board.

The order of the pulse wave velocity recordings was randomized to account for any potential interference between the two measures. cfPWV was obtained using an automatic vascular screening device (VP-1000 Plus, Omron Healthcare, Kyoto, Japan) as previously described (167). Briefly, carotid and femoral arterial pressure waveforms were acquired by applanation tonometry sensors attached on the right common carotid artery (via neck collar) and right femoral artery (via elastic tape around the hip). The distance between common carotid artery and the suprasternal notch was subtracted from the carotid–femoral length.

Heart-thigh pulse wave velocity (htPWV) was measured with the automated vascular screening device (Vasera, Fukuda Denshi, Tokyo, Japan) (100) modified for the measurement of htPWV. A segmometer was used to measure the two major segments of the body for htPWV length (i) from the left sternal border of the second intercostal space to the right femoral artery pulsating site (ii) from the right femoral artery to the center of the thigh cuff. The participants rested in a supine position for at least 10 minutes with the deflated blood pressure cuffs placed on upper arm and upper thigh. In addition, electrocardiogram and phonocardiogram were monitored during this resting period. htPWV was calculated as Lht/(Tb + Tbt), where Lht is the body surface distance from heart to thigh cuff, Tb is the time delay between the second heart sound on phonocardiogram and dicrotic notch on the brachial waveform, and Tbt is the time delay between brachial artery and thigh cuff pulse. In this device, Tb was measured from the time delay between the second heart sound and the dicrotic notch at the brachial artery

under the assumption that these Tb delays are equivalent (161). The foot of the femoral and brachial pulses was detected using the volume displacement waveform measured by two cuffs placed around the upper thigh and proximal to the antecubital fossa, respectively.

Data analyses were performed using the Statistical Package for the Social Sciences version 22 (SPSS, IBM Corp., Armonk, NY). Statistical differences between different methods were evaluated by the Student's t-tests for paired data. Associations of interest were analyzed by Pearson correlational analyses and stepwise regression analyses. The statistical procedure proposed by Bland and Altman was used to compare two different methods (14). First, the correlation and regression analyses were conducted between measured values. The initial step served as the evaluation of the degree of agreement between the two methodologies. Second, the relative differences within each pair of measurements were plotted against the mean of the pair. A p value < 0.05 was used to determine statistical significance.

Table 2. Participant Characteristics

Variable	***Means ± SD***
Age, years	34 ± 16
Males/Females, n	28 M / 22 F
Height, cm	171 ± 10
Weight, kg	73 ±15
BMI, kg/m^2	25 ± 4
Systolic BP, mmHg	121 ± 13
Mean BP, mmHg	88 ± 9
Diastolic BP, mmHg	72 ± 8

BMI = body mass index; BP = blood pressure.

RESULTS

Mean ± SD age, height, body weight, body mass index, and systolic/diastolic blood pressure of the participants were 34 ± 16 years, 171 ± 10 cm, 73.3 ± 15.0 kg, 24.9 ± 4.0 kg/ m^2, and 121 ± 13/72 ± 8 mm Hg, respectively (Table 2). Mean values of cfPWV and htPWV were 713 ± 145 and 699 ± 150 cm/s and were not significantly different (P = 0.43). The mean ± SD difference between the two methods was 14 ± 125 cm/s. Both cfPWV and htPWV were significantly associated with age (r = 0.80 and 0.58), body mass index (r = 0.44 and 0.31), and systolic blood pressure (r = 0.42 and 0.41) (all P < 0.05). Stepwise regression analyses revealed that the strongest correlate for both cfPWV and htPWV was age, explaining 64% and 34% of the respective variances. The two PWV measures demonstrated a strong linear association with a Pearson correlation coefficient of 0.64 (P < 0.001) (Figure 2; panel A) although the regression line deviated from the line of identity. This agreement was consistent with the results of the Bland–Altman plot (Figure 2; panel B).

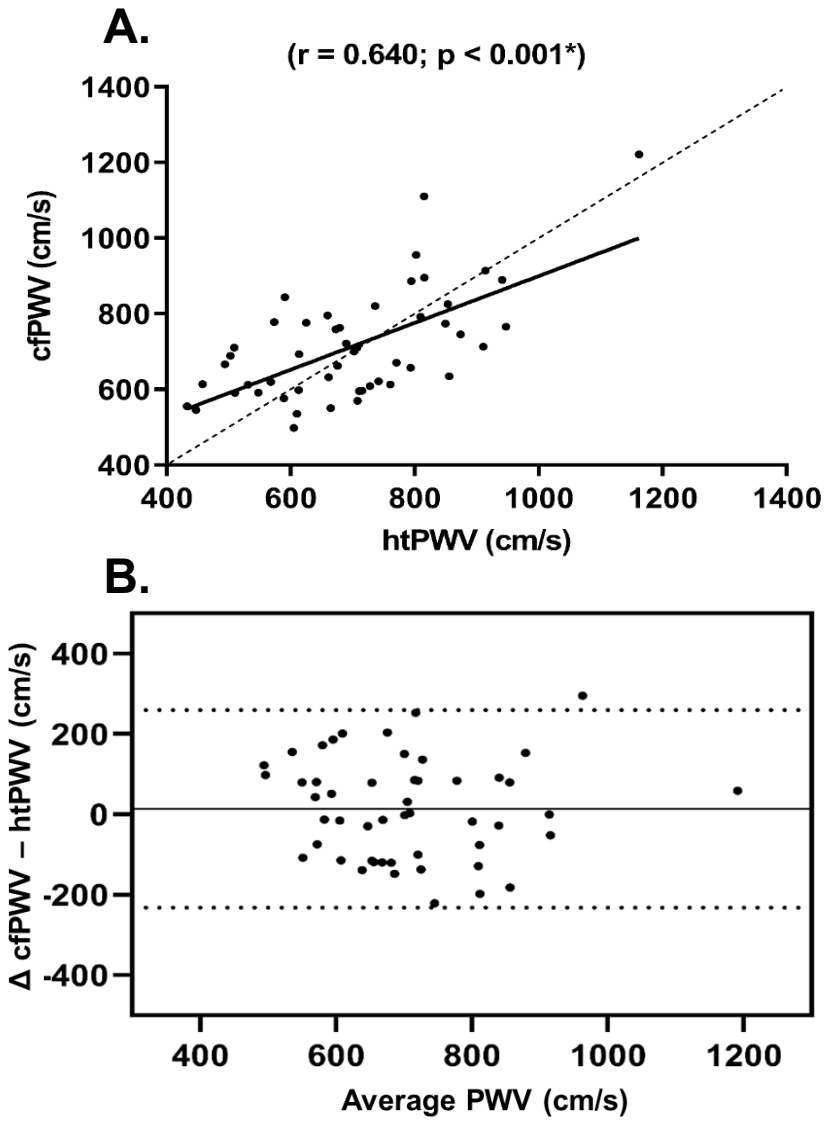

Figure 2. Association between carotid-femoral pulse wave velocity (cfPWV) and heart-thigh pulse wave velocity (htPWV). Pearson correlational analysis is shown in panel A and Bland-Altman plot with mean difference ±2 SDs is displayed in panel B.

DISCUSSION

In the present study, the novel automatic device for measuring arterial stiffness (htPWV) was evaluated by comparing it with the reference standard measure of arterial stiffness using cfPWV. The significant correlation and the small mean differences observed in the Bland–Altman plot reflect a good agreement between the two methods. These results suggest that the automatic noninvasive method, which permits the data acquisition with minimum technical skill, time, and intrusion in an operator-independent fashion, has potential as a screening device for assessing arterial stiffness.

Despite the accumulating evidence indicating the clinical importance of arterial stiffness, this measure has not been incorporated widely in the routine clinical settings. For the measure to be widely adopted, the device would likely need to be straightforward to set-up. The novel device evaluated in the present study uses a combination of electrocardiogram and phonocardiogram to represent the proximal pulse wave. In the cfPWV measurement, the proximal pulse wave is acquired via proper applanation of the carotid artery, which requires technical skills and is often difficult to obtain in some participants including those who are obese. In addition, the use of a thigh cuff and the oscillometric method eliminate the exposure of the inguinal area and the subsequent application of a pressure transducer on the femoral artery. This novel technique is easy, quick, and operator independent.

Although htPWV assessed in the present study is significantly associated with cfPWV, the regression line deviated from the line of identity. Similarly, the correlation with age was much higher with cfPWV ($r = 0.80$) than with htPWV ($r = 0.58$). This may be likely due to the use of thigh cuff to measure femoral pulse waves as this has been reported in

the cuff-based approaches (21, 57). Indeed, when we measured heart-femoral PWV, rather than heart-thigh PWV using the femoral transducer, the correlation with cfPWV was much stronger at r = 0.89 (data not shown). In addition, heart-femoral PWV was associated with age (r = 0.83) similar to cfPWV. Nevertheless, the present results indicate a close association with the reference standard measure of arterial stiffness (cfPWV) and should support the implementation of this technique into a clinical setting.

CHAPTER V. STUDY 3: SIMULATED FREE DIVING: INTERACTION OF EXERCISE AND MULTIPLE REFLEXES

Abstract

Purpose: Free diving evokes a complex cardiovascular response and is accompanied by apnea and exercise. The degrees of diving bradycardia and hypertension induced by the diving reflex are substantial despite the vigorous underwater swimming. This condition provides a circulatory challenge to properly buffer and cushion cardiac pulsations. We determined hemodynamic changes that occur in the vasculature during the diving maneuver. Methods: A total of 20 apparently healthy young participants (26 ± 3 years; 10 men and 10 women) participated in this study. Carotid compliance and hemodynamics were measured during apnea, face immersion in cold water (10°C) and simulated free diving by having participants perform face immersion in cold water while exercising on a cycle ergometer. Results: Simulated free diving resulted in bradycardia ($p<0.001$), increased mean arterial blood pressure ($p<0.001$), increased total peripheral resistance ($p<0.001$), increased arterial compliance ($p=0.007$), increased carotid blood flow velocity ($p<0.001$), as well as decreased cardiac output ($p<0.001$), and decreased ß-stiffness index ($p<0.001$). Conclusion: Carotid arterial compliance is augmented during simulated diving to help buffer cardiac pulsations.

INTRODUCTION

Free diving evokes a complex cardiovascular response known as the diving reflex that is initiated by cold water immersion. The diving reflex is characterized by bradycardia, decreased cardiac output, peripheral vasoconstriction, and increased arterial blood pressure (6). The respiratory physiologist Scholander famously termed this powerful response as “the master switch of life” that acts to preserve oxygen and maintain perfusion to vital organs (142).

The degrees of diving bradycardia and hypertension induced by the diving reflex are fairly substantial in spite of the vigorous underwater swimming performed by sea divers (97). For example, blood pressure has been recorded to reach values as high as 280/200 mmHg (systolic/diastolic) while free diving due to the associated peripheral vasoconstriction and hydrostatic pressure (43). This condition provides a circulatory challenge to properly buffer and cushion cardiac pulsations. The central elastic arteries play an important role in the compliance function. In fact, diving mammals (e.g., seals, sea lions, whales) have a well-adapted arterial structure with substantially larger ascending aorta relative to the descending or thoracic aorta to help buffer cardiac pulsations and maintain perfusion during substantial bradycardia due to vagal stimulation that occurs with the diving reflex (145). Additionally, professional Japanese female pearl divers (Ama) demonstrate lower systemic arterial stiffness and superior reservoir function in central elastic arteries (159, 168).

The hemodynamic changes that occur in the vasculature during the diving maneuver have not yet been extensively investigated because the diving maneuver evokes very complex physiological responses. In addition to the diving reflex that is

triggered by facial immersion in cold water, diving maneuvers are accompanied by apnea and exercise. Each of these stimuli have varying influences on the cardiovascular responses during the diving maneuver (46). Blood flow velocity in the carotid artery is increased during the diving reflex (121). Additionally, middle cerebral artery blood flow velocity increases with facial cooling and apnea (2). Currently, there is no information available regarding how arterial compliance changes in response to diving maneuver as well as components of diving maneuver including apnea, face immersion, and exercise. Therefore, the purpose of this study was to comprehensively determine the cardiovascular responses that occur during the simulated free diving. We hypothesized that carotid arterial compliance would be augmented during the simulated free diving (diving reflex with exercise) when compared to exercise, apnea, and facial immersion individually to help buffer cardiac pulsations.

METHODS

Twenty apparently healthy young participants participated in this study (Table 3). Participants came to the laboratory on two occasions. Participants submitted their informed consent and completed health history questionnaire when first arriving to the laboratory. Health status and lifestyle habits of the participants were obtained using the Health Research Questionnaire. Then participants had their height and body weight recorded. The first visit was used as a familiarization session as a combination of apnea, face immersion, and exercise was very challenging. During the second visit, following a 4 hour fast, data collection was conducted. After at least 15 minutes of supine rest, arterial stiffness was assessed. Afterwards, participants were transferred to the cycle ergometer

and carotid arterial compliance, heart rate, blood pressure, and cardiac output were measured for 20 seconds at rest. We then measured arterial and cardiac functions during the conditions of exercise, apnea, face immersion in cold water (10°C) without apnea (using a snorkel device), and simulated free diving, all during steady state exercise for 20 seconds. Specifically, we combined all the conditions to simulate free diving by having participants perform face immersion in cold water with apnea while exercising on a cycle ergometer with simultaneous measurements of their cardiovascular responses. Specifically, to simulate the diving reflex in the laboratory setting, participants were placed on a cycle ergometer and maintained exercise at a light to moderate intensity (25-50 W) while holding their breath (for 20 seconds) with facial immersion in cold water (10°C). These parameters were based on pilot testing, as trained divers have been shown to exercise at 80 W with breath holds of 40 seconds as previously described (6). Participants completed each condition (exercise alone [EX], apnea with exercise [EX+A], facial immersion while breathing using a snorkel and exercise [EX+FI], and simulated free diving, which included facial immersion with apnea and exercise [EX+A+FI]) with simultaneous measurements of carotid diameter and pressure, blood pressure, heart rate, oxygen saturation, cardiac output, and total peripheral resistance responses being recorded for 20 seconds after steady state exercise was reached prior to the beginning of each experimental condition. Participants were allowed to rest for up to 5 minutes between each condition. On average, each condition was preceded by about 5 minutes of exercise to allow heart rate and blood pressure to reach steady state. All procedures were reviewed and approved by The University of Texas at Austin’s Institutional Review Board.

Arterial stiffness was measured as previously described in our laboratory (83). Briefly, cardio-ankle vascular index (CAVI) and heart-thigh (htPWV) pulse wave velocity were measured using an automated vascular screening device (Vasera, Fukuda Denshi, Tokyo, Japan). Blood pressure cuffs were applied to the ankles, thighs, and upper arms. CAVI is calculated using an electrocardiogram, phonocardiogram, brachial artery, and ankle artery waveforms by measuring PWV via dividing vascular length by the time taken for the pulse wave to travel from the aortic valve to the ankles (148, 192). CAVI was calculated by incorporating the stiffness parameter β, which has been reported to be a blood pressure-independent measure of arterial stiffness.

Arterial compliance was measured noninvasively during each condition as previously described in our laboratory (139). Carotid artery diameter was measured from images derived from an ultrasound machine (iE33 Ultrasound System, Phillips, Bothel, WA) equipped with a high-resolution linear-array transducer using duplex mode. A longitudinal image of the cephalic portion of the common carotid artery was acquired 1–2 cm proximal to the carotid bulb with the transducer placed at 90° to the vessel. Doppler ultrasound was used to acquire blood flow velocity in the carotid artery with an insonation angle of 60 degrees. The carotid diameters were measured utilizing automated image analysis software (Carotid Analyzer, Medical Imaging Applications, Coralville, IA). Arterial pressure waveforms were obtained using an arterial tonometry placed on the contralateral carotid artery and recorded on a data acquisition software (Windaq 2000, Dataq Instruments, Akron, OH). Carotid artery compliance, cross-sectional compliance, distensibility coefficient, ß-stiffness index, and carotid pulse wave velocity were calculated as previously described (33, 177).

Arterial compliance (m/kPa)

$$\frac{\triangle D}{\triangle P}$$

Cross-sectional compliance (m^2/kPa)

$$\frac{\pi(2D_d \cdot \Delta D + \Delta D^2)}{4\Delta P}$$

Distensibility coefficient (1/kPa)

$$\frac{(2D_d \cdot \Delta D + \Delta D^2)}{\Delta P \cdot D_d{}^2}$$

ß-stiffness index (AU)

$$\ln\left(\frac{\text{Systolic BP}}{\text{Diastolic BP}}\right) \cdot \frac{D_d}{\Delta D}$$

Carotid PWV (m/s)

$$\sqrt{\frac{1}{\rho DC}}$$

Arterial blood pressure and heart rate responses were measured continuously and noninvasively using the PortaPres finger plethysmograph device (Finapres Medical Systems BV, Amsterdam, Netherlands) as previously described by our laboratory (106). Specifically, systolic, diastolic and mean blood pressure were measured continuously throughout the testing procedures using beat-by-beat finger plethysmography. The plethysmographic cuff was placed around the middle phalanx of the finger, and the modulated cuff pressure maintained transmural pressure at an effective zero. Prior to the exercise, the finger blood pressure was calibrated using brachial blood pressure. The beat-by-beat results were subsequently analyzed using the Beatscope software

(Finapres Medical Systems BV, Amsterdam, Netherlands), which also calculated cardiac output using the validated Model Flow method and total peripheral resistance.

A pulse oximeter (Nellcor N-20P, Colin Corporation, Hayashi Komaki, Japan) was used to measure oxygen saturation of arterial hemoglobin noninvasively. This sensor was placed on the index finger of each participant during all conditions.

Data analysis was performed using the Statistical Package for the Social Sciences version 22 (SPSS, IBM Corp., Armonk, NY). Normality of the data was confirmed with a Shapiro-Wilk test. A repeated measures analysis of variance was utilized to compare changes from baseline of each dependent variable to changes during each of the maneuvers. The Greenhouse-Geisser correction of degrees of freedom was used when sphericity assumptions were violated. Significant effects were further analyzed with Bonferroni post hoc comparisons. A priori power analysis has been conducted using the program G*Power (version 3.1.9.2). With an effect size of 0.3 and power of .90, only 19 participants would have been required to reach statical significance for each of our dependent variables. As such, the overall sample size of 20 participants in this study achieved adequate power (>80%). A p-value of <0.05 was used to determine statistical significance.

Table 3. Selected Participant Characteristics

Variable	***Means ± SD***
Age, years	26 ± 3
Males/Females, n	10 / 10
Height, cm	174 ± 10
Body weight, kg	71 ± 14
BMI, kg/m^2	23 ± 3
Heart rate, bpm	60 ± 12
Systolic BP, mmHg	117 ± 10
Mean BP, mmHg	87 ± 6
Diastolic BP, mmHg	70 ± 7
htPWV, m/s	6.5 ± 1.8
CAVI	6.0 ± 0.5
Ankle Brachial Index	1.06 ± 0.1

BMI = body mass index; BP = blood pressure;

htPWV = heart thigh pulse wave velocity;

CAVI = cardio-ankle vascular index.

RESULTS

Participant Characteristics and Cardiovascular Measures

The participant characteristics are presented in Table 3. Experimental data are presented in Table 4. A significant increase in heart rate was observed with exercise, apnea, and facial immersion when compared with rest ($F_{[1,19]} = 16.405$, $p<0.001$; Figure 3). However, simulated diving (the diving reflex) brought the heart rate down to resting levels ($p = 0.124$). Oxygen saturation did not change with any experimental conditions ($p = 0.190$). Mean blood pressure was significantly elevated during all conditions ($F_{[1,19]} = 34.311$, $p<0.001$; Figure 5), with a further elevation observed during simulated diving when compared with exercise ($p<0.001$), apnea ($p=0.016$), and facial immersion ($p<0.001$). Stroke volume did not change significantly with any condition ($F_{[1,19]} = 2.521$, $p=0.091$; Figure 3). Cardiac output increased with all conditions ($F_{[1,19]} = 16.844$, $p<0.001$; Figure 3), with an attenuated response during simulated diving when compared with exercise and facial immersion ($p=0.002$; $p=0.026$, respectively). Total peripheral resistance was attenuated during exercise and facial immersion when compared with rest ($F_{[1,19]} = 8.139$, $p<0.001$; Figure 3). Additionally, total peripheral resistance was augmented during simulated diving when compared with exercise ($p<0.001$), apnea ($p=0.008$), and facial immersion ($p=0.003$).

Carotid Artery Measurements

Carotid pulse pressure increased with all experimental conditions when compared with rest ($F_{[1,19]} = 17.180$, $p<0.001$; Figure 5). Arterial compliance increased with simulated diving when compared with rest ($F_{[1,19]} = 9.109$, $p=0.007$; Figure 4), and was augmented when compared with exercise and apnea ($p=0.007$; $p<0.001$, respectively).

Cross-sectional compliance increased with all conditions ($F = _{[1,19]}$ 27.525, $p<0.001$) with a greater increase observed during simulated diving compared to exercise ($p<0.001$), apnea ($p<0.001$), and facial immersion ($p=0.003$). Facial immersion also augmented cross-sectional compliance more than exercise ($p<0.001$). Distensibility coefficient increased during simulated diving when compared with rest ($F = _{[1,19]}$ 4.197, $p=0.023$), and was greater during simulated diving when compared with exercise and apnea ($p = 0.008$; $p<0.001$, respectively). β-stiffness index decreased with each condition ($F = _{[1,19]}$ 10.127, $p < 0.001$; Figure 4), with the greatest decrease observed during simulated diving when compared to exercise ($p<0.001$), apnea ($p<0.001$), and facial immersion ($p=0.015$). Carotid pulse wave velocity decreased during simulated diving when compared with rest ($F = _{[1,19]} = 4.087$, $p=0.001$), and was lower when compared to exercise and apnea ($p=0.002$; $p<0.001$, respectively). Lastly, carotid blood flow velocity increased during all conditions ($F = _{[1,19]}$ 9.801, $p<0.001$; Figure 5), with an augmented response during simulated diving when compared to exercise and apnea ($p=0.008$; $p=0.001$, respectively).

Moreover, since there was an even number of male and female participants an exploratory analysis was conducted to determine any sex differences in the main outcome variables. There was no significant interaction effect for condition by sex for arterial compliance ($F = _{[1,19]}$ 2.058, $p=0.118$), cross-sectional compliance ($F = _{[1,19]}$ 0.315, $p=0.793$), distensibility coefficient ($F = _{[1,19]}$ 2.420, $p=0.081$), and β-stiffness index ($F = _{[1,19]}$ 1.446, $p=0.247$).

Table 4. Cardiovascular Responses to Experimental Conditions

Variable	*Rest*	*EX*	*EX+A*	*EX+FI*	*EX+A+FI*	*p-value*
Heart Rate, bpm	76 ± 4	105 ± 5*†	92 ± 7*†	99 ± 6*†	85 ± 7	**<0.001**
Oxygen Saturation, %	97.8 ± 0.3	97.8 ± 0.4	97.5 ± 0.4	97.7 ± 0.3	97.3 ± 0.4	0.190
Mean Blood Pressure, mmHg	91 ± 4	107 ± 4*†	122 ± 4*†	115 ± 6*†	132 ± 5*	**<0.001**
Stroke Volume, mL/beat	81 ± 5	92 ± 6	97 ± 7	94 ± 6	98 ± 11	0.091
Cardiac Output, L/min	5.8 ± 0.2	9.3 ± 0.5*†	8.6 ± 0.6*	8.9 ± 0.5*†	7.5 ± 0.6*	**<0.001**
Total Peripheral Resistance, dynes/sec/cm^{-5}	1343 ± 94	951 ± 72*†	1253 ± 147†	1140 ± 101*†	1624 ± 186	**<0.001**
Carotid Pulse Pressure, mmHg	40 ± 2	61 ± 3*	68 ± 4*	61 ± 4*	69 ± 4*	**<0.001**
Arterial Compliance, m/kPa x 10^{-4}	1.6 ± 0.1	1.5 ± 0.1†	1.4 ± 0.1†	1.8 ± 0.2	2.0 ± 0.1*	**0.007**
Cross-sectional Compliance, m^2/kPa x 10^{-5}	3.2 ± 0.2	4.5 ± 0.3*†	4.8 ± 0.3*†	5.6 ± 0.4*†	7.3 ± 0.5*	**<0.001**
Distensibility Coefficient, 1/kPa x 10^{-2}	5.3 ± 0.4	5.3 ± 0.5†	4.8 ± 0.3†	6.2 ± 0.6	6.9 ± 0.5*	**0.023**
β-stiffness Index	4.8 ± 0.4	3.9 ± 0.3*†	3.7 ± 0.2*†	3.3 ± 0.3*†	2.5 ± 0.2*	**<0.001**
Carotid Pulse Wave Velocity, m/s	4.4 ± 0.2	4.4 ± 0.1†	4.7 ± 0.2†	4.2 ± 0.2	3.8 ± 0.1*	**0.011**
Carotid Blood Flow Velocity, cm/s	92 ± 3	103 ± 4*†	104 ± 4*†	114 ± 5*	117 ± 5*	**<0.001**

Data are means ± SEM. *p<0.05 vs. Rest, †p<0.05 vs. Simulated Diving (EX+A+FI)
EX= Exercise; A= Apnea; FI= Facial Immersion.

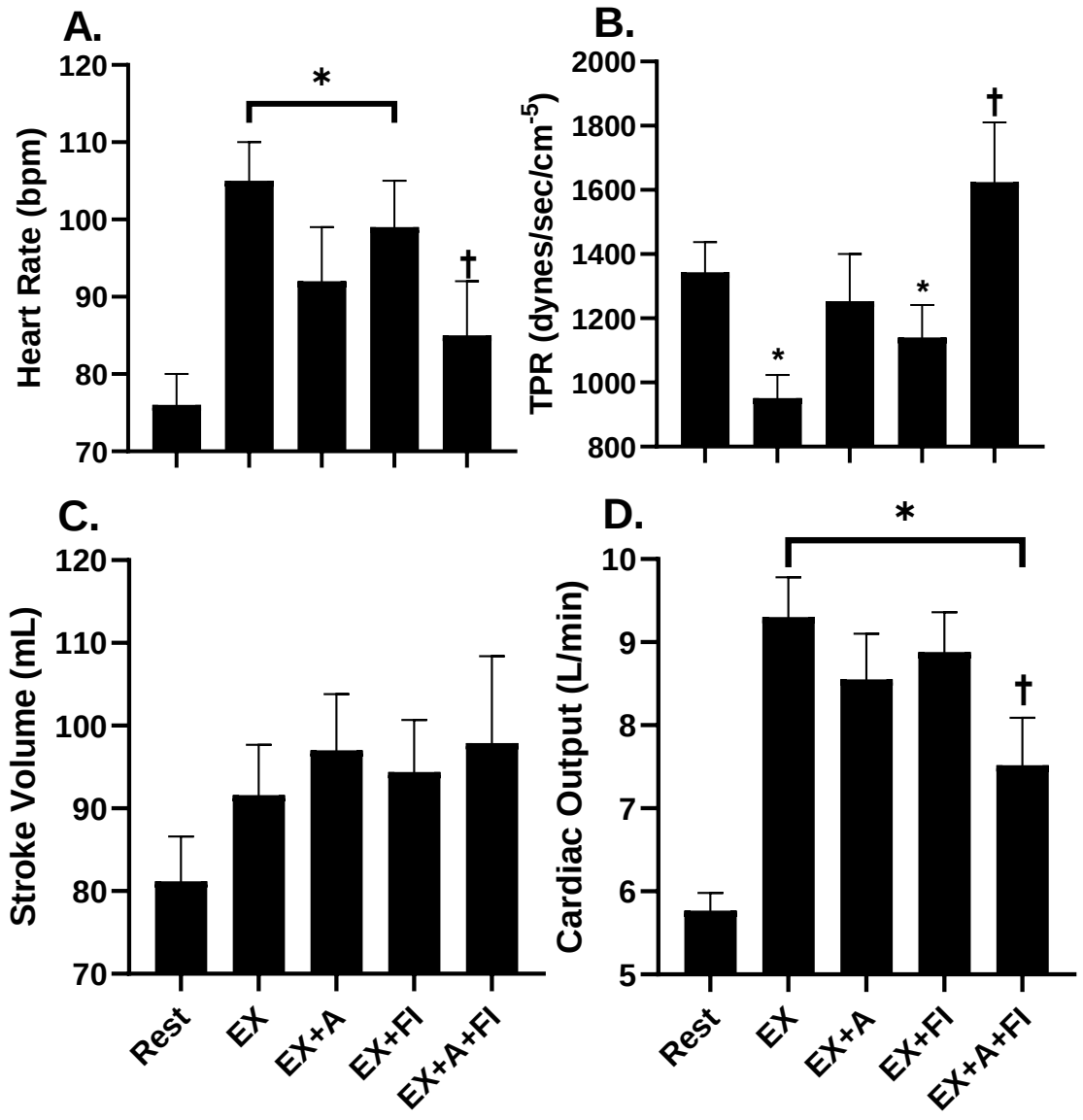

Figure 3. The heart rate (panel A), total peripheral resistance (TPR; panel B), stroke volume (panel C), and cardiac output (panel D) response to each experimental condition (EX= Exercise; A= Apnea; FI= Facial Immersion). *p<0.05 vs. Rest. †p<0.05 vs. EX, EX+A, and EX+FI.

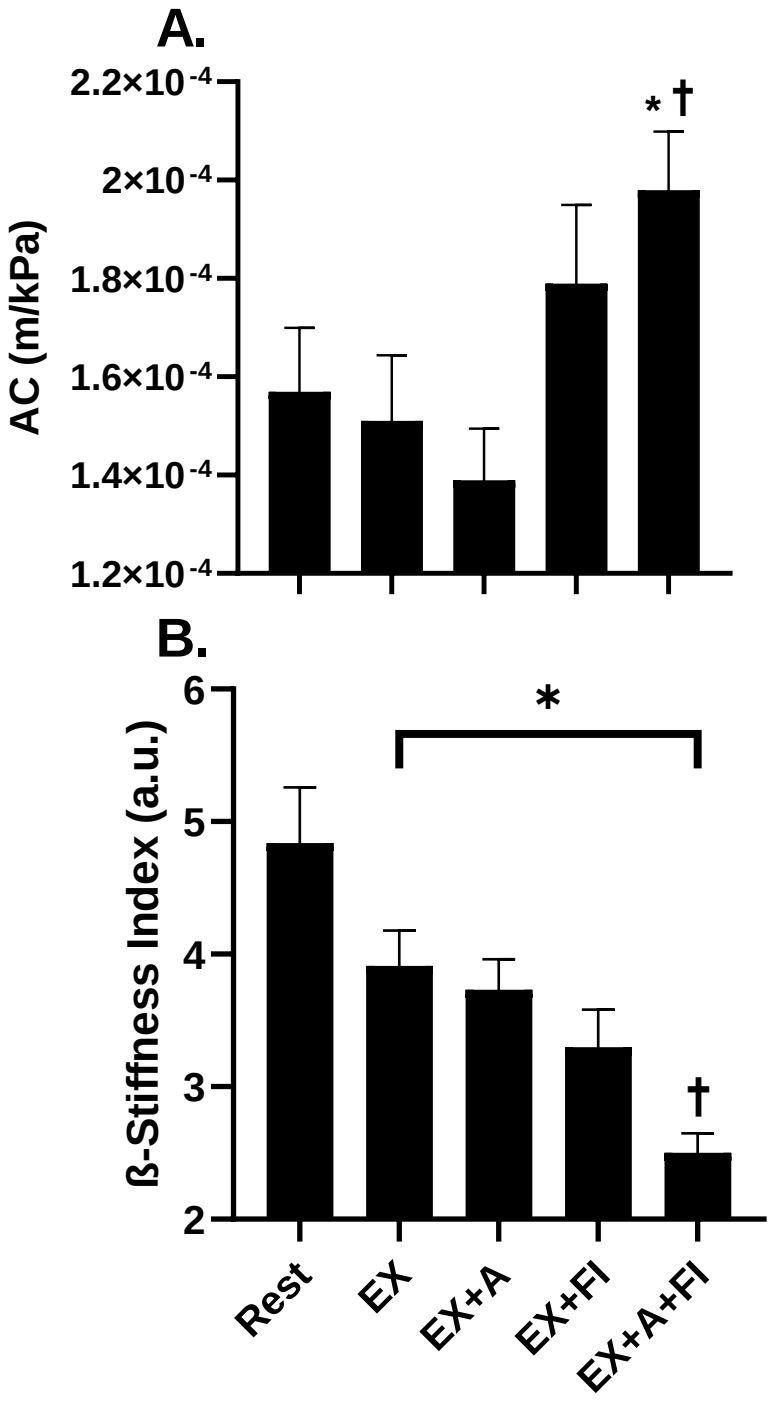

Figure 4. The arterial compliance (AC; panel A), and β-stiffness index (panel B) response to each experimental condition (EX= Exercise; A= Apnea; FI= Facial Immersion). *p<0.05 vs. Rest. †p<0.05 vs. EX, EX+A, and EX+FI.

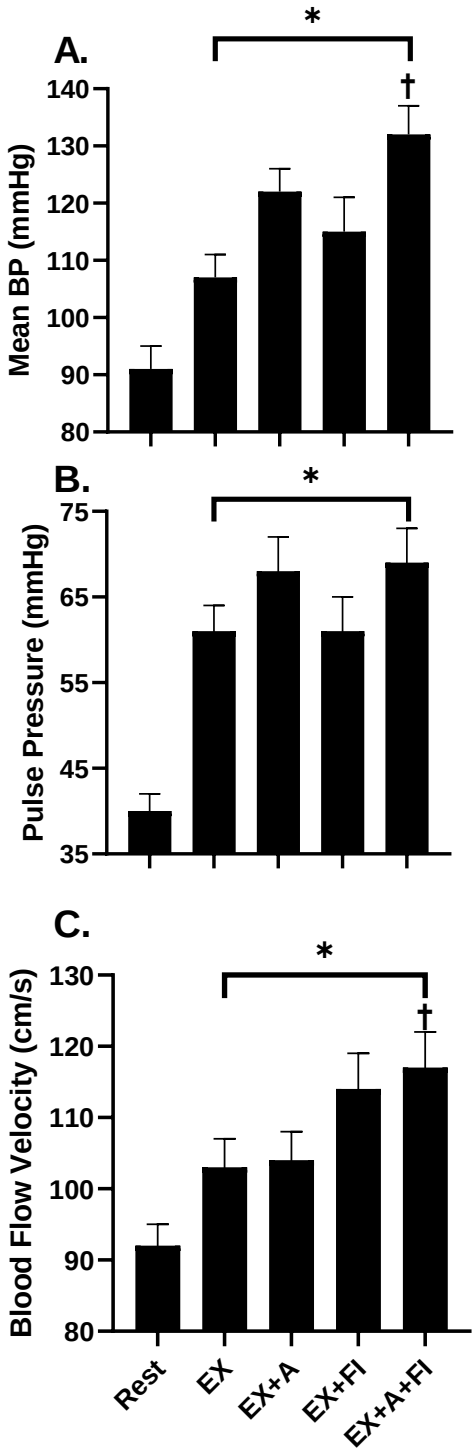

Figure 5. The mean blood pressure (panel A), carotid pulse pressure (panel B), and carotid blood flow velocity (panel C) response to each experimental condition (EX= Exercise; A= Apnea; FI= Facial Immersion). *$p<0.05$ vs. Rest. †$p<0.05$ vs. EX, EX+A, and EX+FI.

DISCUSSION

The main aim of this study was to investigate how simulated diving would mediate arterial function. To the best of our knowledge, this is the first study to demonstrate that carotid arterial compliance is augmented during simulated free diving, likely to help buffer and cushion increased arterial pressure waves. Specifically, we found increases in arterial compliance, cross-sectional compliance, and distensibility coefficient with simulated diving when compared with rest. Importantly, these effects were mostly augmented above separate conditions of exercise, apnea, and facial immersion. We also demonstrated decreased arterial stiffness (the inverse of arterial compliance) via β-stiffness index and carotid pulse wave velocity. Additionally, our study reproduces previous findings that carotid blood flow velocity increases during the diving reflex (121).

The simulated diving in the present study displayed similar cardiovascular responses previously observed with the diving reflex. Specifically, the bradycardia during simulated free diving is similar to the decreases in heart rate demonstrated with the original studies performed in pearl divers (78, 141). Previous research has demonstrated that mean arterial blood pressure is increased during free diving, which we observed with our participants (13, 43). Additionally, total peripheral resistance increased during simulated diving when compared with exercise, indicating a sympathetically-mediated peripheral vasoconstriction response (13, 64, 171). Cardiac output has been demonstrated to not change with the diving reflex (171). Although we observed an increase in cardiac output from rest with simulated diving (due to the superimposed exercise), there was a decrease in cardiac output when compared with exercise alone. As such, cardiac output is decreased via bradycardia to conserve oxygen during

simulated diving (13, 95). Previous research demonstrated that stroke volume increases during free diving due to a central shift of blood flow with hydrostatic pressure, which increases cardiac pre-load (97). However, we did not observe a significant increase in stroke volume in our simulated diving, likely due to the lack of full body water immersion and the associated hydrostatic pressure that occurs with increased diving depths.

Exercise

To isolate the effects of exercise on the cardiovascular responses from simulated diving on our outcome variables, we compared the responses between simulated diving and exercise alone. Of course, exercise increases demand on the cardiovascular system to meet the blood flow requirements to the working muscles. Autonomic control of cardiovascular responses during exercise are primarily mediated by central command, the exercise pressor reflex, the arterial baroreflex, cardiopulmonary baroreceptors, and arterial chemoreceptors (44). By comparing simulated diving to the exercise stimulus, we can infer how the diving reflex contributes to the cardiovascular responses. Importantly, acute exercise has been demonstrated to increase arterial compliance (92, 115). We effectively demonstrated an augmented response in arterial compliance with simulated diving when compared with exercise alone.

Apnea

Apnea produces enough stimulus to trigger a decrease in heart rate, although bradycardia is blunted during apnea alone when compared with facial immersion and apnea together (143, 158). During breath holding, there is increased anaerobic metabolism as increases in plasma lactate concentration are observed (6). During apnea,

there is also reduced oxygen uptake from the lungs to the blood, thereby conserving lung oxygen reserve (86). Hypercapnia has been demonstrated with apnea (6), which elevates blood pressure via chemoreceptor stimulation. We found that apnea did not influence arterial compliance or arterial stiffness any more than exercise. However, simulated diving enhanced these responses when compared to apnea and exercise.

Facial Immersion

Additionally, we wanted to isolate the effect of facial immersion in cold water (10ºC) from apnea by providing a snorkeling device to allow breathing. Facial immersion in cold water triggers a neuronal afferent response from the trigeminal nerve (3). The nerve fibers innervating the anterior nasal mucosa and paranasal region are essential for this reflex via chemesthetic trigeminal chemoreceptors (103). These afferent neuronal signals are transmitted to the brainstem, resulting in efferent neuronal signals that activate the sympathetic and parasympathetic nervous systems (muscarinic M2 receptors and the vagus nerve), which induce peripheral vasoconstriction and bradycardia, respectively. Indeed, facial immersion in cold water without apnea resulted in bradycardia due to increased vagal tone and increased total peripheral resistance when compared with the exercise condition. Moreover, facial immersion increased cross-sectional compliance more than exercise. Since arterial compliance accounts for the change in blood pressure on the distensibility of the artery, we can assume a withdrawal of sympathetic tone on the carotid artery is responsible for the increased compliance during facial immersion (166). Indeed, arterial compliance has previously been shown to decrease with elevated levels of muscle sympathetic nerve activity independent of blood pressure changes (59).

Notably, simulated diving enhanced these responses (cross-sectional compliance) when compared to facial immersion.

This study is not without limitations. First, our experimental conditions were not randomized. However, we allowed the participants to rest between each condition until each cardiovascular measure was brought back down to baseline. Second, a cold pressor test could have been conducted to control for the influence of cold stress on the cardiovascular responses. Although, facial immersion in cold water could be perceived as a cold pressor test, this condition was superimposed with exercise. Lastly, we were not able to control for the potential effects of hypercapnia during apnea. We are unsure if the 20 seconds of apnea superimposed on exercise resulted in a buildup of arterial carbon dioxide in our study, as previous research with 40 seconds of apnea superimposed on exercise has demonstrated hypercapnia (6).

In conclusion, our present study found that simulated free diving results in bradycardia, increased mean arterial blood pressure, increased total peripheral resistance, increased arterial compliance, increased carotid blood flow velocity, as well as decreased cardiac output and decreased atrial stiffness. These extensive cardiovascular adjustments aid in redistributing blood flow centrally while buffering pulsatile blood flow. These findings are significant because it contributes to scientific knowledge about the influence of interaction of multiple stimuli on the cardiovascular system’s blood flow redistribution and regulation during simulated free diving. Ultimately, such knowledge helps us further understand how the diving reflex preserves oxygen and perfuses vital organs.

CHAPTER VI: SUMMARY & FUTURE DIRECTIONS

Typically, blood pressure is used in a clinical setting to determine patients' risk for developing cardiovascular disease. However, there are several noninvasive measurements of vascular function that provide prognostic utility. These measurements include endothelial function, arterial stiffness, and arterial compliance. For example, a 1% increase of FMD results in an 8-13% decreased risk of cardiovascular events. While a 1 m/s increase cfPWV is associated with a 7% increased risk of cardiovascular events. Similarly, decreased arterial compliance increases risk of end-organ damage (e.g., heart, brain). As such, this dissertation aimed to demonstrate how each of the noninvasive measurements of vascular function could be used to conduct an intervention, assess clinical feasibility, and a physiological investigation.

The first study demonstrated that 24-hour ambulatory blood pressure monitoring did not influence endothelial function via ischemic preconditioning. These findings have implications in that ambulatory blood pressure monitoring and FMD could be measured in tandem with no interference, as this is common practice in many cardiovascular research studies. The second study demonstrated that a novel and easy-to-use measurement of central arterial stiffness, htPWV, has good agreement with the reference standard for arterial stiffness, cfPWV. Although arterial stiffness is an excellent predictor for future cardiovascular events, it has not been widely used in clinical settings due to it technical nature. This study's findings support the used of htPWV in clinical settings due its potential diagnostic utility. Lastly, the third study demonstrated that simulated free diving results in bradycardia, increased blood pressure, increased total peripheral resistance, increased carotid blood flow velocity, and decreased cardiac output. More

importantly, carotid arterial compliance was significantly elevated and arterial stiffness decreased during the simulated diving maneuver. These findings contribute to scientific knowledge about the interaction of multiple stimuli on the cardiovascular system's blood flow redistribution and regulation during simulated free diving helping to preserve oxygen and perfuse vital organs.

Future studies should investigate if longer durations of ambulatory blood pressure monitoring influence endothelial function. Additionally, determine how effective of a predictor htPWV is for cardiovascular events when compared to cfPWV in large population-based studies. Finally, verify the results of this study on how arterial function is modulated during free diving in the sea utilizing innovative technologies that provide data acquisition in such a challenging environment. In conjunction with our current findings the additional studies would demonstrate how noninvasive measures of vascular function are useful for researchers and clinicians.

CHAPTER VII. APPENDICES

Appendix A: Study 1 Informed Consent

IRB USE ONLY
Study Number: 2017-07-0043
Approval Date: 9/12/2017
Expires: 9/11/2018

Consent for Participation in Research

Title: 24-hour blood pressure measurements and ischemic conditioning

Introduction

The purpose of this form is to provide you information that may affect your decision as to whether or not to participate in this research study. The person performing the research will answer any of your questions. Read the information below and ask any questions you might have before deciding whether or not to take part. If you decide to be involved in this study, this form will be used to record your consent.

Purpose of the Study

You have been asked to participate in a research study about the relationship between 24-hour blood pressure monitoring and vascular function. The purpose of this study is to determine if wearing a blood pressure monitor for 24-hours a day would change vascular function.

A description of this study will be available on http://www.ClinicalTrials.gov as required by U.S. Law. This web site will not include information that can identify you. At most, the web site will include a summary of the results. You can search this web site at any time.

What will you be asked to do?

If you agree to participate in this study, you will be asked to wear a blood pressure monitor for 24 hours (even when you are sleeping). Before and after wearing the blood pressure monitor, we will assess vascular function in the research laboratory. This study will take a total of 26 hours to complete (24 hours of blood pressure monitoring and 1 hour each of vascular function testing before and after the blood pressure monitoring) and will include approximately 30 study participants.

Health questionnaire will determine if you are eligible to participate and to get a general sense of your overall health status. If you are found to be eligible to participate in the study based on the questionnaire, we will perform several measurements described below.

Vascular function will be tested by inflating a cuff that is placed on the arm to block blood flow to the hand for 5 minutes. During these tests, an ultrasound probe will be placed on the skin of your arm. Your body's response to the stress will be measured (by the ultrasound probe placed on the arm) before and after the cuff is released.

24-hour blood pressure monitor will be programmed to record the blood pressure from 6 am to 11 pm every 15 minutes and from 11 pm to 6 am every 20 minutes. You will take this blood pressure monitor home and return it the next day.

When you come back to the laboratory to return the blood pressure monitor, we will repeat the vascular function test above.

NOTE:
This is a research study and, therefore, not intended to provide a medical or therapeutic diagnosis or treatment. The intervention provided in the course of this study is not necessarily

equivalent to the standard method of prevention, diagnosis, or treatment of a health condition.

What are the risks involved in this study?

This research may involve risks that are currently unforeseeable. Possible risks associated with this study are temporary discomfort (numbness and/or tingling) at the fingertip during and after cuff inflation during the vascular function tests. These symptoms usually disappear within 30 seconds following cuff deflation.

What are the possible benefits of this study?

You will receive no direct benefit from participating in this study. However, the possible benefits of participation are measurements of 24-hour blood pressure and vascular function free of charge. Or you may not gain any benefit at all.

Do you have to participate?

No, your participation is voluntary. You may decide not to participate at all or, if you start the study, you may withdraw at any time. Withdrawal or refusing to participate will not affect your relationship with The University of Texas at Austin (University) in anyway.

If you would like to participate, please sign this informed consent form and return it to the investigator. You will receive a copy of this form.

Will there be any compensation?

You will not receive any type of payment participating in this study.

What if you are injured because of the study?

In the event of a research-related injury, it is important that you notify the Principal Investigator of the research-related injury immediately. You and/or your insurance company or health care plan may be responsible for any charges related to research-related injuries. Compensation for an injury resulting from your participation in this research is not available from The University of Texas at Austin. However, you are not waiving any of your legal rights by participating in this study.

How will your privacy and confidentiality be protected if you participate in this research study?

Any information that is obtained in this study and that can be identified with you will remain confidential and will be released only with your permission. Your responses will not be linked to your name in any written or verbal report of this research project. The researchers might use information learned from this study in scientific journal articles or in presentations. None of this information will identify you personally.

If it becomes necessary for the Institutional Review Board to review the study records, information that can be linked to you will be protected to the extent permitted by law. Your research records will not be released without your consent unless required by law or a court order. The data resulting from your participation may be made available to other researchers in the future for research purposes not detailed within this consent form. In these cases, the data will contain no identifying information that could associate it with you, or with your participation in any study.

Whom to contact with questions about the study?

Prior, during or after your participation you can contact the researcher Hirofumi Tanaka at 512 232-4801 or send an email to htanaka@austin.utexas.edu for any questions or if you feel th you have been harmed.

This study has been reviewed and approved by The University Institutional Review Board and study number is **2017-07-0043.**

Whom to contact with questions concerning your rights as a research participant?

Whom to contact with questions concerning your rights as a research participant?

For questions about your rights or any dissatisfaction with any part of this study, you can cont anonymously if you wish, the Institutional Review Board by phone at (512) 471-8871 or emai orsc@uts.cc.utexas.edu.

Participation

If you agree to participate, you will bring the signed forms to BEL 842 during your initial visit prior to beginning the study. Or you may fill them out at the lab (BEL 842) prior to beginning the study.

Signature

You have been informed about this study's purpose, procedures, possible benefits and risks, and you have received a copy of this form. You have been given the opportunity to ask questions before you sign, and you have been told that you can ask other questions at any time. You voluntarily agree to participate in this study. By signing this form, you are not waiving any of your legal rights.

_____

Printed Name

_____ _____

Signature Date

As a representative of this study, I have explained the purpose, procedures, benefits, and the risks involved in this research study.

_____

Print Name of Person obtaining consent

_____ _____

Signature of Person obtaining consent Date

Appendix B: Study 2 Informed Consent

IRB USE ONLY
Study Number: 2018-05-0118
Approval Date: 09/05/2018
Expires: 07/12/2019

Consent for Participation in Research

Title: Reproducibility and Validity of Arterial Stiffness Measurements

Introduction

The purpose of this form is to provide you information that may affect your decision as to whether or not to participate in this research study. The person performing the research will answer any of your questions. Read the information below and ask any questions you might have before deciding whether or not to take part. If you decide to be involved in this study, this form will be used to record your consent.

Purpose of the Study

You have been asked to participate in a research study to evaluate the reproducibility and validity of measurement methodologies of arterial stiffness. To accomplish this aim, we will compare values obtained for arterial stiffness from an automatic device to those measured using gold standard methodologies (manual methods). This measurement is often used by doctors to screen for risks of future cardiovascular disease and stroke.

A description of this study will be available on http://www.ClinicalTrials.gov as required by U.S. Law. This web site will not include information that can identify you. At most, the web site will include a summary of the results. You can search this web site at any time.

What will you be asked to do?

If you agree to participate, you will be asked to participate in one-day testing that lasts ~2 hours. During the testing period, measurements described below will be made on you. All the measurements will be conducted in the Cardiovascular Aging Research Laboratory (room 842) in Bellmont Hall. Before each of the visits you will be asked to fast for 4 hours, not have alcohol or caffeine within 12 hours, and abstain from strenuous physical activity for 24 hours prior to each visit.

- Health questionnaire will determine if you are eligible to participate and to get a general sense of your overall health status. If you are found to be eligible to participate in the study based on the questionnaire, we will perform several measurements described below.

- Body Measurements of your height and body weight will be assessed on a physician's balance scale.

- Blood pressure will be measured non-invasively in the laboratory via standard automated blood pressure cuff that would be found in a Doctor's office.

- Arterial Stiffness will be measured **noninvasively** by placing either a painless instrument called a Doppler probe or a pencil-like transducer (depending on the location) on the skin over the throat, carotid (neck) and common femoral (hip

joint) arteries. This measurement is based on experiments showing that the faster your blood pressure travels, the stiffer your arteries are. We will perform this measurement using two different methods as follows.

- First, automatic blood pressure cuffs will be placed on your upper arms and ankles. The cuffs will then inflate and slowly deflate to measure your arterial stiffness.
- Next, we will repeat the same procedure manually; we will use hand-held probes or transducers on the skin.

All assessments of vascular function will take place in the research laboratory. This study will take a total of ~2 hours to complete and will include approximately 50 study participants.

NOTE:
This is a research study and, therefore, not intended to provide a medical or therapeutic diagnosis or treatment. The intervention provided in the course of this study is not necessarily equivalent to the standard method of prevention, diagnosis, or treatment of a health condition.

What are the risks involved in this study?

Every effort has been made by the investigators to keep the risk and discomfort involved in this study to a minimum. Nevertheless, the potential risks associated with this study include a slight risk of discomfort associated with the placement of blood pressure cuffs at high pressure, and a slight risk of fainting associated with the placement of a probe over the carotid (neck) artery.

What are the possible benefits of this study?

You will receive no direct benefit from participating in this study. However, the possible benefits of participation are measurements of blood pressure, heart rate and vascular function. Additionally, your participation will contribute to scientific knowledge that is likely to result in the establishment of validity for an automatic device to screen for risks associated with future cardiovascular disease in men and women.

Do you have to participate?

No, your participation is voluntary. You may decide not to participate at all or, if you start the study, you may withdraw at any time. Withdrawal or refusing to participate will not affect your relationship with The University of Texas at Austin (University) in anyway.

If you would like to participate, please sign this informed consent form and return it to the investigator. You will receive a copy of this form.

Will there be any compensation?

No compensation will be provided for participating in this study.

What if you are injured because of the study?

In the event of a research-related injury, it is important that you notify the Principal Investigator of the research-related injury immediately. You and/or your insurance company or health care plan may be responsible for any charges related to research-related injuries. Compensation for an injury resulting from your participation in this research is not available from The University of Texas at Austin. However, you are not waiving any of your legal rights by participating in this study.

How will your privacy and confidentiality be protected if you participate in this research study?

Any information that is obtained in this study and that can be identified with you will remain confidential and will be released only with your permission. Your responses will not be linked to your name in any written or verbal report of this research project. The researchers might use information learned from this study in scientific journal articles or in presentations. None of this information will identify you personally.

If it becomes necessary for the Institutional Review Board to review the study records, information that can be linked to you will be protected to the extent permitted by law. Your research records will not be released without your consent unless required by law or a court order. The data resulting from your participation may be made available to other researchers in the future for research purposes not detailed within this consent form. In these cases, the data will contain no identifying information that could associate it with you, or with your participation in any study.

Whom to contact with questions about the study?

Prior, during or after your participation you can contact the researcher Hirofumi Tanaka at 512-232-4801 or send an email to htanaka@austin.utexas.edu for any questions or if you feel that you have been harmed.

This study has been reviewed and approved by The University Institutional Review Board and the study number can be given to you by the research investigator at any time upon request.

Whom to contact with questions concerning your rights as a research participant?

For questions about your rights or any dissatisfaction with any part of this study, you can contact, anonymously if you wish, the Institutional Review Board by phone at (512) 471-8871 or email at orsc@uts.cc.utexas.edu.

Participation

If you agree to participate, you will bring the signed forms to BEL 842 during your initial visit prior to beginning the study. Or you may fill them out at the lab (BEL 842) prior to beginning the study.

Signature

You have been informed about this study's purpose, procedures, possible benefits and risks, and you have received a copy of this form. You have been given the opportunity to ask questions before you sign, and you have been told that you can ask other questions at any time. You voluntarily agree to participate in this study. By signing this form, you are not waiving any of your legal rights.

_____
Printed Name

_____ _____
Signature Date

As a representative of this study, I have explained the purpose, procedures, benefits, and the risks involved in this research study.

_____
Print Name of Person obtaining consent

_____ _____
Signature of Person obtaining consent Date

Appendix C: Study 3 Informed Consent

Title of the Project: Interaction of Exercise and Multiple Reflexes
Principal Investigator: Brandon Fico, M.S., The University of Texas at Austin
Faculty Advisor: Hirofumi Tanaka, Ph.D., The University of Texas at Austin
Study Sponsor: No sponsor

Consent to Participate in Research

Invitation to be Part of a Research Study

You are invited to be part of a research study. This consent form will help you choose whether or not to participate in the study. Feel free to ask if anything is not clear in this consent form.

Important Information about this Research Study

Things you should know:

- The purpose of the study is to determine the cardiovascular responses during a simulated diving maneuver.
- In order to participate, you must be a healthy adult between the ages of 18 and 45 years old.
- If you choose to participate, you will be asked to exercise at light to moderate intensity on a cycle ergometer while holding your breath (apnea) with face immersion in cold water and without apnea (10°C/ 50°F). Each breath hold will last up to a maximum of 30 seconds. During this simulated diving maneuver, a few cardiovascular measurements will be recorded. This will take two visits lasting up to 2 hours each.
- Risks or discomforts from this research include fatigue, syncope (fainting), shortness of breath, elevated blood pressure, and skeletal muscle cramping during exercise.
- There are no direct benefits for participating in the study. However, you will receive blood pressure and arterial stiffness assessments.
- Taking part in this research study is voluntary. You do not have to participate, and you can stop at any time.

More detailed information may be described later in this form.

Please take time to read this entire form and ask questions before deciding whether to take part in this research study.

What is the study about and why are we doing it?

You have been asked to participate in a research study investigating the effect of free diving on the cardiovascular systems regulation of blood flow and redistribution. The purpose of this study is to determine if arterial compliance increases during the diving maneuver.

What will happen if you take part in this study?

If you agree to take part in this study, you will be asked to come to the laboratory on two occasions following a 4 hour fast. After at least 15 minutes of supine rest, arterial stiffness and

compliance will be assessed non-invasively. We will then measure arterial and cardiac function during the conditions of apnea, face immersion in cold water with and without apnea, and exercise. Additionally, we will combine all the conditions to simulate free diving by conducting face immersion in cold water (10°C/50°F) with apnea while exercising on a cycle ergometer with simultaneous measurements of your cardiovascular responses. Below are the measurements to be taken in detail.

Health questionnaire will determine if you are eligible to participate and to get a general sense of your overall health status. If you are found to be eligible to participate in the study based on the questionnaire, we will perform several measurements described below.

Arterial stiffness will be tested by placing blood pressure cuffs on your arms, thighs, and ankles. Additionally, electrocardiogram leads will be placed on your arms and a phonocardiogram (microphone) will be placed on your chest to listen for the closing of your heart valves. The transit time in which it takes the pressure waveforms to travel from proximal to distal cuffs will determine the degree of arterial stiffness.

Arterial compliance will be measured by placing an ultrasound transducer and tonometer (pressure sensor) on your neck. The ultrasound probe will be placed on one side of your neck and the presser sensor will be placed on the other side and will remain in place throughout the testing.

Blood pressure and heart rate will be measured by placing a cuff on your middle finger. The cuff will be inflated to an effective zero (slight pressure) and will measure changes in your blood pressure and heart rate throughout the testing.

Oxygen saturation will be measured by placing a sensor on your index finger. This device uses light absorption to measure your oxygen saturation of arterial hemoglobin.

How long will you be in this study and how many people will be in the study?

Participation in this study requires 2 visits, each lasting no more than 2 hours.

What risks and discomforts might you experience from being in this study?

There are some risks you might experience from being in this study. They include fatigue, syncope (fainting), shortness of breath, elevated blood pressure, and skeletal muscle cramping during exercise. The potential risks of the proposed studies will be minimized by: (a) using only safe, well-established procedures; (b) constant, personal monitoring of each experimental session by investigators that have training and experience using the experimental equipment; (c) the appropriate clinical supervision and availability of emergency equipment; (d) employing complete confidentiality of the record keeping process to be employed. In the case of emergency, standard laboratory procedure to call 911 will be performed. The researchers will let you know about any significant new findings (such as additional risks or discomforts) that might make you change your mind about participating in this study.

How could you benefit from this study?

There are no direct benefits for participation in this study. However, from being in this study you will receive blood pressure and arterial stiffness assessments. Or you may not gain any benefit at all. Although, societal benefits include the advancement of how the cardiovascular system responds under stress (e.g. the diving reflex) which may be useful in emergency medicine.

What will happen to the samples and/or data we collect from you?

As part of this study we will collect non-invasive cardiovascular measures during the experimental procedures. These measurements will be presented as group average responses for research presentations and publications. Any information that is obtained in this study and that can be identified with you will remain confidential and will be released only with your permission. Your responses will not be linked to your name in any written or verbal report of this research project. The researchers might use information learned from this study in scientific journal articles or in presentations. None of this information will identify you personally.

How will we protect your information?

We will protect your information by storing your information in a locked file cabinet in the P.I.'s office. Additionally, data will be stored by code, and at no time will any individual data point be identified with a particular individual participant. This procedure is applicable to electronic data as well. Your name and any other information that can directly identify you will be stored separately from the data collected as part of the project**.** The data or samples that we will collect about you will not be shared with any other researchers. We plan to publish the results of this study. To protect your privacy, we will not include any information that could directly identify you.

Information about you may be given to the following organizations:

- Representatives of UT Austin and the UT Austin Institutional Review Board

What will happen to the information we collect about you after the study is over?

Your name and other information that can directly identify you will be deleted from the research data collected as part of the project. The data resulting from your participation may be made available to other researchers in the future for research purposes not detailed within this consent form. In these cases, the data will contain no identifying information that could associate it with you, or with your participation in any study.

What if we learn something about your health that you did not know?

As part of this study, we may learn medically relevant information about you. If we learn something that you and your doctor did not know, we will ask you to follow-up with your physician.

How will we compensate you for being part of the study?

You will not receive any type of payment for your participation.

Who will pay if you are hurt during the study?

In the event of a research-related injury, it is important that you notify the Principal Investigator of the research-related injury immediately. You and/or your insurance company or health care plan may be responsible for any charges related to research-related injuries. Compensation for an injury resulting from your participation in this research is not available from The University of Texas at Austin. You are not waiving any of your legal rights by participating in this study.

What are the costs to you to be part of the study?

To participate in the research, you will need not need to pay for anything (e.g. a parking pass will be provided).

Your Participation in this Study is Voluntary

It is totally up to you to decide to be in this research study. Participating in this study is voluntary. Your decision to participate will not affect your relationship with The University of Texas at Austin. You will not lose any benefits or rights you already had if you decide not to participate. Even if you decide to be part of the study now, you may change your mind and stop at any time. You do not have to answer any questions you do not want to answer.

If you decide to withdraw before this study is completed, your data will be deleted.

Is it possible that you will be asked to leave the study?

You may be asked to leave the study if it is determined by the research team that it is unsafe for you to continue. If any of the following issues come up, we will have to ask you to stop participating: fainting, nausea, or an excessive drop in blood pressure.

Contact Information for the Study Team

If you have any questions about this research, you may contact:

Brandon Fico
Phone: (512)471-8594
Email: bfico@utexas.edu

Dr. Hirofumi Tanaka
Phone: (512)232-4801
Email: htanaka@austin.utexas.edu

Contact Information for Questions about Your Rights as a Research Participant

If you have questions about your rights as a research participant, or wish to obtain information, ask questions, or discuss any concerns about this study with someone other than the researcher(s), please contact the following:

The University of Texas at Austin Institutional Review Board
Phone: 512-232-1543
Email: irb@austin.utexas.edu
Please reference study number 2019-11-0014.

Your Consent

By signing this document, you are agreeing to be in this study. We will give you a copy of this document for your records. We will keep a copy with the study records. If you have any questions about the study after you sign this document, you can contact the study team using the information provided above.

I understand what the study is about and my questions so far have been answered. I agree to take part in this study.

_____
Printed Subject Name

_____
Signature Date

Appendix D: Medical/Health History Questionnaire

Medical/Health Questionnaire

Cardiovascular Aging Research Laboratory
University of Texas at Austin

Personal Information

Today's Date _____ Subject ID _____

Please circle the highest grade in school you have completed:

Elementary school	1	2	3	4	5	6	7	8
High school	9	10	11	12				
College/Post Grad	13	14	15	16	17	18	19	20+

What is your marital status? ☐ Single ☐ Married; ☐ Widowed ☐ Divorced; Separated

Ethnic Background: ☐ Hispanic or Latino ☐ Not Hispanic or Latino

Race:

☐ White ☐ American Indian/Alaskan Native ☐ Pacific Islander

☐ Black or African American ☐ Asian

Symptoms or Signs Suggestive of Disease

Check appropriate box:

Yes	No		
☐	☐	1.	Have you experienced unusual pain or discomfort in your check, neck, jaw, arms or other areas that may be due to heart problems?
☐	☐	2.	Have you experienced unusual fatigue or shortness of breath at rest, during usual activities, or during mild-to-moderate exercise (e.g., climbing stairs, carrying groceries, brisk walking, cycling)?
☐	☐	3.	When you stand up, or sometimes during the night while you are sleeping, do you have difficulty breathing?
☐	☐	4.	Do you lose your balance because of dizziness or do you ever lose consciousness?
☐	☐	5.	Do you suffer from swelling of the ankles (ankle edema)?
☐	☐	6.	Have you experienced an unusual and rapid throbbing or fluttering of the heart?
☐	☐	7.	Have you experienced severe pain in your leg muscles during walking?
☐	☐	8.	Has a doctor told you that you have a heart murmur?

Chronic Disease Risk Factors

Check appropriate box:

Yes	No		
☐	☐	9a.	Are you a male over age 45 years or a female over age 55 years?
☐	☐		b. Are you a female who has experienced premature menopause?
☐	☐		c. If you answered "yes" to 9b, are you on estrogen replacement therapy?
☐	☐	10.	Has your father or brother had a heart attack or died suddenly of heart disease before the age of 55; has your mother or sister experienced these heart problems before the age of 65?

Yes No

☐ ☐ 11. Are you a current cigarette smoker?
☐ ☐ 12. Has a doctor told you that you have high blood pressure (more than 140/90 mm Hg) or a heart condition?
☐ ☐ 13. Is your total serum cholesterol greater than 200 mg/dl, or has a doctor told you that your cholesterol is at a high risk-level?
☐ ☐ 14. Do you have diabetes mellitus?
☐ ☐ 15. Are you physically inactive and sedentary (little physical activity on the job or during leisure time)?
☐ ☐ 16. Do you have a bone or joint problem that could be made worse by a change in your physical activity?
☐ ☐ 17. During the past year, would you say that you have experienced enough stress, strain, and pressure to have a significant effect on your health?
☐ ☐ 18. Do you eat foods nearly every day that are high in fat and cholesterol such as fatty meats, cheese, fried foods, butter, whole milk, or eggs?
☐ ☐ 19. Do you weigh 30 or more pounds than you should?
☐ ☐ 20. Do you know of any other reason you should not do physical activity?

Medical History

21. *Please check which of the following conditions you have had or now have. Also check medical conditions in your family (father, mother, brother(s), or sister(s)). Check as many as apply.*

Self	Family	Medical Condition	Self	Family	Medical Condition
☐	☐	Coronary heart disease, heart attack; by-pass surgery	☐	☐	Major injury/fracture to foot, leg knee
☐	☐	Arrhythmias	☐	☐	Major injury to back or neck
☐	☐	Angina	☐	☐	Major injury/fracture to hip or shoulder
☐	☐	High blood pressure	☐	☐	Rheumatoid Arthritis
☐	☐	Peripheral vascular disease	☐	☐	Osteoarthritis
☐	☐	Phlebitis or emboli	☐	☐	Gout
☐	☐	Other heart problems	☐	☐	Osteoporosis
☐	☐	Stroke	☐	☐	Fibromyalgia
☐	☐	Asthma	☐	☐	Diabetes mellitus
☐	☐	Bronchitis	☐	☐	Kidney disease
☐	☐	COPD (emphysema)	☐	☐	Cataracts
☐	☐	Lung cancer	☐	☐	Glaucoma
☐	☐	Breast cancer	☐	☐	Hearing loss
☐	☐	Prostate cancer	☐	☐	Depression
☐	☐	Skin cancer	☐	☐	Anxiety, phobias
☐	☐	Colorectal cancer	☐	☐	Eating disorders
☐	☐	Other cancer. Specify:	☐	☐	Sleeping problems
☐	☐	Gallstones/gallbladder disease	☐	☐	Substance abuse problems (alcohol, other drugs, etc.)
☐	☐	Liver disease (cirrhosis)	☐	☐	Chronic Fatigue Syndrome
☐	☐	Hepatitis	☐	☐	Thyroid problems

Self	Family	Medical Condition	Self	Family	Medical Condition
☐	☐	Anemia (low iron)	☐	☐	Hysterectomy
☐	☐	Stomach/duodenal ulcer	☐	☐	Problems with menstruation
☐	☐	Rectal growth or bleeding	☐	☐	Post-menopausal (date:)
☐	☐	Crohne's disease	☐	☐	Raynaud's disease
☐	☐	Irritable bowel syndrome	☐	☐	Allergies
☐	☐	Marfan's syndrome			

Any other health problems. Please specify and include information on any recent illnesses, hospitalizations, or surgical procedures.

22. *Please check any of the following medications you take regularly and give the name of the medication.*

Medication	**Name of Medication**
☐ Heart medicine	_____
☐ Blood pressure medicine	_____
☐ Blood cholesterol medicine	_____
☐ Hormones	_____
☐ Birth control medicine	_____
☐ Medicine for breathing/lungs	_____
☐ Insulin	_____
☐ Other medicine for diabetes	_____
☐ Arthritis medicine	_____
☐ Medicine for depression	_____
☐ Medicine for anxiety	_____
☐ Thyroid medicine	_____
☐ Medicine for ulcers	_____
☐ Painkiller medicine	_____
☐ Allergy medicine	_____
☐ Other (please specify)	_____
☐ Do you have any drug allergies?	_____
☐ Dietary supplements (please specify)	_____

Body Weight

23. What is the most you have ever weighed? _____ pounds

24. Are you now trying to:

☐ Lose weight ☐ Gain weight ☐ Stay about the same ☐ Not trying to do anything

Stress

25. During the past month, how would you rate your overall level of stress?

☐ Very high ☐ High ☐ Moderate ☐ Low

26. In the past year, how much effect has stress had on your health?

☐ A lot ☐ Some ☐ Hardly any or none

27. On average, how many hours of sleep do you get in a 24-hour period?

☐ Less than 5 ☐ 5-6.9 ☐ 7-9 ☐ More than 9

Substance Use

28. How would you describe your cigarette smoking habits?
  ☐ Never smoked
  ☐ Used to smoke. How many years has it been since you smoked? _____ years
  ☐ Still smoke. How many cigarettes a day do you smoke on average? ___cigarettes/day

29. How many alcoholic drinks do you consume? (A "drink" is a glass of wine, a wine cooler, a 16oz bottle/12oz can of beer, a shot glass of liquor, or a mixed drink).

☐ Never use alcohol ☐ Less than 1 per week ☐ 1-6 per week ☐ 1 per day
☐ 2-3 per day ☐ More than 3 per day

30. In one sitting, how many drinks do you typically consume? _____
31. How many cups (8 ounces) of coffee do you drink per day? _____
32. How many ounces of sodas containing caffeine do you drink per day? _____

Physical Fitness, Physical Activity/Exercise

33. Considering a **7-Day period** (a week), how many times on the average do you do the following kinds of exercise for **more than 15 minutes** during your **free time** (write on each line the appropriate number).

a) **STRENUOUS EXERCISE (HEART BEATS RAPIDLY)** **Times Per Week**
(i.e. running, jogging, hockey, football, soccer, squash, basketball, cross country skiing, judo, roller skating, vigorous swimming, vigorous long distance bicycling) _____

b) **MODERATE EXERCISE (NOT EXHAUSTING)**
(i.e. fast walking, baseball, tennis, easy bicycling, volleyball, badminton, easy swimming, alpine skiing, popular and folk dancing) _____

c) **MILD EXERCISE (MINIMAL EFFORT)**
(i.e. yoga, archery, fishing from river bank, bowling, horseshoes, golf, snow-mobiling, easy walking) _____

34. Considering a 7-Day period (a week), during your leisure-time, how often do you engage in any regular activity long enough to work up a sweat (heart beats rapidly)

☐ OFTEN ☐ SOMETIMES ☐ NEVER/RARELY

35. How long have you exercised or played sports regularly?
  ☐ I do not exercise regularly ☐ Less than 1 year ☐ 1-2 years
  ☐ 2-5 years ☐ 5-10 years ☐ More than 10 years

Occupational Health

36. Please describe your main job title and duties.

_____

37. How much hard physical work is required on your job?
☐ A great deal ☐ A moderate amount ☐ A little ☐ None

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